R_{GN} 0.91;[26] $[\alpha]D$ +1° (2 min) → −3° (1 hr, final) (*c* 0.6, H_2O); ir, 3600–2500-cm^{-1} region (broad), 1590 and 1510 cm^{-1} (medium peaks), and 1150, 1105, 1070, and 1035 cm^{-1} (with increasing intensities in that order).

Anal. Calcd for $C_6H_{16}Cl_2N_2O_4$ (251.1): C, 28.70; H, 6.42. Found: C, 28.55; H, 7.00.

Methyl 2-Acetamido-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-mannopyranoside (16).—A small amount of **16** had been obtained previously ("isomer **6**," mp 223–224°),[8] but no analytical and rotation data had been recorded and no configuration assigned. A larger quantity of **16** was now prepared. A number of fractions of methyl 2-amino-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-hexopyranosides (from the earlier work) which were rich in what is now know to be the D-*manno* isomer (**15**) (then[8] referred to as "isomer **4**") but contained various amounts of the D-*gluco* isomer, were pooled and *N*-acetylated as described.[8] From 1.843 g of material was thus obtained (a) a fraction (330 mg) of sparingly soluble *gluco*-*N*-acetyl derivative, mp 308–310° dec (lit. mp 310–311° dec); (b) a fraction (727 mg) of methanol-soluble **16**, mp 223–225° dec, $[\alpha]D$ −78° (*c* 1, DMF); and (c) an additional fraction of **16** (925 mg), mp 217–218° dec, $[\alpha]D$ −92.5° (*c* 1, DMF). Joint recrystallization of fractions b and c from methanol–ether gave prisms: mp 224–225° dec; $[\alpha]D$ −94.7° (*c* 1.03, DMF).

Anal. Calcd for $C_{16}H_{20}N_2O_7$ (352.3): C, 54.54; H, 5.72; N, 7.95. Found: C, 54.44; H, 5.86; N, 7.84.

Methyl 2-Acetamido-2,3-dideoxy-3-nitro-β-D-mannopyranoside (17).—Compound **16** (500 mg) was heated on a steam bath for 30 min in 70% acetic acid (50 ml). The solution was then evaporated with consecutive additions of toluene, water, and ethanol. The resulting, nearly colorless syrup crystallized in part from ethanol–ethyl acetate–ether, at 5°. The large, prismatic needles (145 mg, mp 90–91°) were recrystallized from the same solvents to give **17**: mp 90°; $[\alpha]D$ −48.5° (*c* 0.8, H_2O); ir, 3500–3100 (broad), 1660 (strong, amide I), and 1550 cm^{-1} (broad, amide II and NO_2).

Anal. Calcd for $C_9H_{16}N_2O_7$ (264.2): C, 40.91; H, 6.10; N, 10.60. Found: C, 40.69; H, 6.23; N, 10.49.

In the above experiment, only 39% of **17** had crystallized. The mother liquor containing the remainder was clarified with charcoal and evaporated to give a syrup which was dissolved in methanol and hydrogenated for 22 hr in the presence of acetic anhydride (0.5 ml) and platinum catalyst [from 200 mg of PtO_2, prehydrogenated in methanol (9 ml) and acetic acid (1 ml)]. The syrupy product obtained on work-up was revealed by tlc (methanol–ethanol–acetone, 1:1:2) to contain a considerable amount of unreduced **17**. The hydrogenation was therefore repeated, and then the product was found to be rich in a slowly moving component although it still contained some faster moving **17**. The slowly moving reduction product (**13**) was isolated in pure form, in a yield of 31 mg, by column chromatography as described for the preparation of **13** from **12**: $[\alpha]D$ −107.5° (*c* 0.65, H_2O); mp 115–119° (undepressed upon admixture of **13** from **12**). It was identical with **13** according to tlc and ir spectra.

Registry No.—**2**, 21870-83-7; **3**, 21899-38-7; **4**, 21870-84-8; **5**, 21870-85-9; **6**, 21870-86-0; **7**, 21870-87-1; **9**, 21870-88-2; **9a**, 21870-89-3; **10**, 21870-90-6; **11**, 21870-91-7; **12**, 21871-05-6; **13**, 21871-06-7; **14**, 21871-07-8; **16**, 21871-08-9; **17**, 21871-09-0.

Acknowledgment.—The authors gratefully acknowledge generous financial support from the Life Insurance Medical Research Fund, Rosemont, Pa. F. Kienzle thanks the Ogilvie Flour Mills Co., Ltd., for a postgraduate fellowship.

Plant Antitumor Agents. IV. An Approach toward the Synthesis of Camptothecin[1,2]

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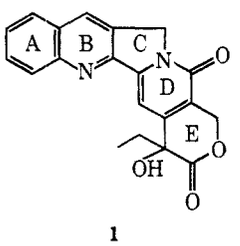
Received April 22, 1969

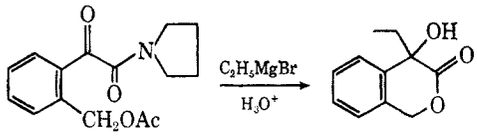
The tricyclic compound **5** containing the A,B,C ring system of camptothecin (**1**) and suitable functionality for constructing the D and E rings has been synthesized. Some of the results obtained in attempts to convert **5** into the aldehyde **6** are described.

A recent communication from this laboratory described the isolation and structure determination of the novel alkaloid camptothecin (**1**).[3] This compound is of interest not only because of its unusual structure but also because of its antitumor and antileukemic activity.[3]



1

It was felt that a successful synthesis of camptothecin would require that formation of the labile α-hydroxy lactone moiety (ring E) be postponed to the final steps. Studies[4] on the model compound **2** indicated that ring E could be prepared from an appropriate α-keto amide by reaction with ethyl magnesium bromide.



2

Accordingly it was decided to prepare 5-acetoxy-4-carboxy-2,2-diethoxypentanoic acid pyrrolidine amide (**3**) which contains suitable functionality for the synthesis of the E ring of camptothecin and a potentially active methylene group (C-3) which would be required for formation of the D ring of camptothecin. The acid **3** could then be coupled with 1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (**4**) to give

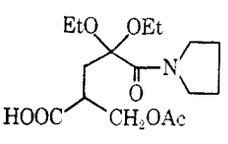
(1) Previous paper in this series: M. E. Wall, H. L. Taylor, L. W. Ambrosio, and K. H. Davis, *J. Pharm. Sci.* in press.

(2) Presented in part at the 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968.

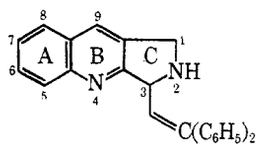
(3) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. I. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **88**, 3888 (1966).

(4) M. E. Wall, F. I. Carroll, J. A. Kepler, M. C. Wani, and M. L. Honjoh, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968, M17.

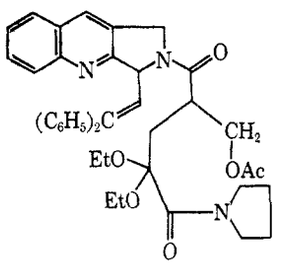
2-(2-acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (**5**). The compound **5** was considered to be an important intermediate in the synthesis of camptothecin since it contains not only the A,B,C, ring system of camptothecin but also possesses functionality which appeared to be suitable for the closure of rings D and E as well. We wish to report at this time the synthesis of **5** and some of the results obtained in attempts to convert **5** into 2-(2-acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-formyl-2H-pyrrolo[3,4-*b*]quinoline (**6**).



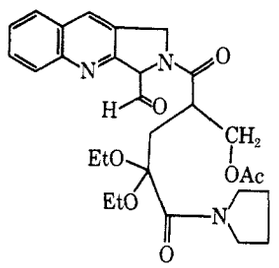
3



4



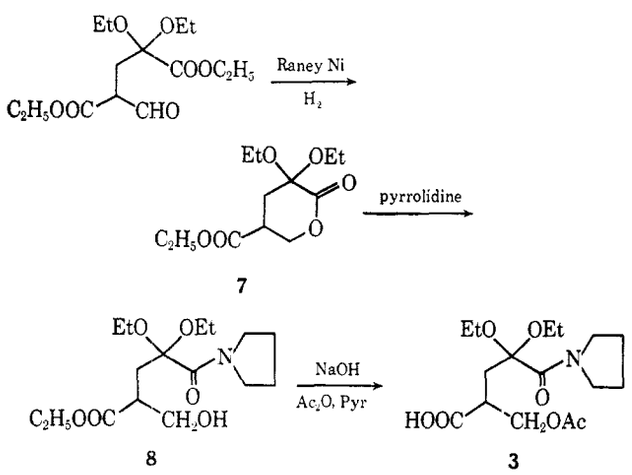
5



6

The acid **3** was prepared according to the precedure outlined in Scheme I. Diethyl 4-formyl-2-oxoglutarate

SCHEME I



diethyl ketal[5] was reduced in the presence of Raney nickel to diethyl 4-hydroxymethyl-2-ketoglutarate diethyl ketal which lactonized upon distillation to give 2,2-diethoxy-4-carbethoxy-5-hydroxypentanoic acid δ-lactone (**7**) (ir 1770 and 1740 cm^{-1}). Treatment of **7** with pyrrolidine resulted in attack exclusively at the lactone carbonyl to give 2,2-diethoxy-4-carbethoxy-5-hydroxypentanoic acid pyrrolidine amide (**8**) (ir 1720 and 1615 cm^{-1}). Basic hydrolysis of **8** followed by acetylation gave the desired carboxylic acid intermediate **3** which had the expected infrared and nmr spectra.

The amine, 1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (**4**) was synthesized as outlined in Scheme II. Ethyl 1-ethoxycarbonyl-3-oxopyrrolidine-2-ylacetate[6] was allowed to react with *o*-aminobenzaldehyde[7] and sodium hydride in benzene to afford, after esterification, 2-carbethoxy-3-carbethoxyethyl-1,3-dihydro-2H-pyrrolo[3,4-*b*]quinoline (**9**). The ultraviolet spectrum of **9** was similar to that of an authentic sample of 2,3-dimethylquinoline.[8] Treatment of **9** with an excess of phenylmagnesium bromide gave 2-carbethoxy-1,3-dihydro-3-(2′,2′-diphenyl-2′-hydroxyethyl)-2H-pyrrolo[3,4-*b*]quinoline (**10**). The alcohol **10** was converted into the cyclic carbamate **11** upon treatment with alcoholic potassium hydroxide. Prolonged treatment of **11** with alcoholic potassium hydroxide resulted in its hydrolysis into 1,3-dihydro-3-(2′,2′-diphenyl-2′-hydroxyethyl)-2H-pyrrolo[3,4-*b*]-quinoline (**12**).

Attempts to form an amide linkage between **12** and **3** in the presence of dicyclohexylcarbodiimide were not successful. It was felt that our failure to achieve coupling at this stage was due to hydrogen bonding between the hydroxyl and the amine functions of **12**. The first attempts to remove this interaction by dehydration of **12** in refluxing benzene and *p*-toluenesulfonic acid were not successful. More vigorous conditions (refluxing toluene) resulted in extensive decomposition. On the other hand, dehydration of alcohol **10** was easily accomplished in refluxing benzene and *p*-toluenesulfonic acid giving 1,3-dihydro-2-carbethoxy-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (**13**); however, all attempts to remove the N-carbethoxy protecting group from this compound to give **4** resulted either in no reaction or complete decomposition. The desired amino olefin **4** was best prepared as the hydrobromide by the action of cold 48% hydrobromic acid on the cyclic carbamate **11**. The amino alcohol **12** could also be dehydrated by the action of 48% hydrobromic to give **4** but in inferior yields. The amino olefin **4** was used immediately for the preparation of the amide **5** without further purification because of its instability.

The intermediate **5** was prepared from **3** and **4** using dicyclohexylcarbodiimide. The results of several experiments indicated that the best yields of **5** were obtained when the reaction was carried out at high concentration in purified dichloromethane. Two diastereomers of **5** were obtained, only one of which could be crystallized. The spectral properties of the two isomers were nearly identical and confirmed the proposed structure.[9]

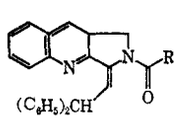
(5) H. Plieninger, G. Ege, R. Fischer, and W. Hoffmann, *Chem. Ber.*, **94**, 2114 (1961).

(6) J. W. Clark-Lewis and P. I. Mortimer, *J. Chem. Soc.*, 189 (1961).

(7) L. I. Smith and J. W. Opie, *Org. Syn.*, **28**, 11 (1948).

(8) J. Eliasberg and P. Friedlander, *Chem. Ber.*, **25**, 1752 (1892).

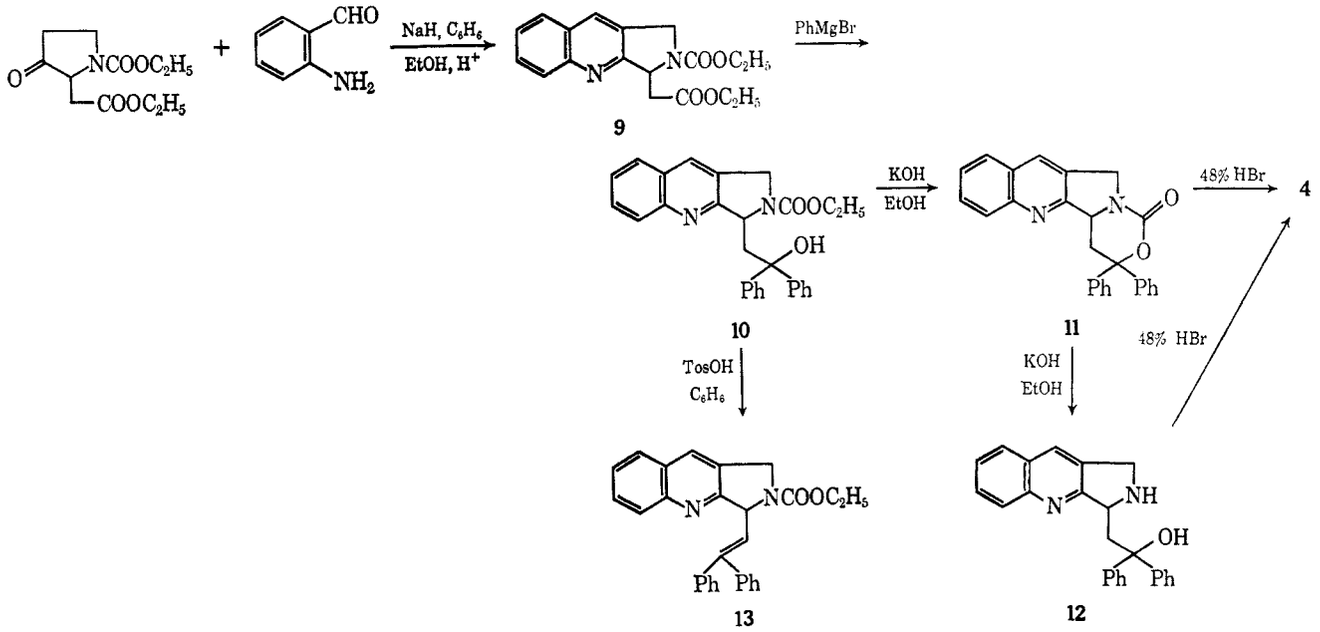
(9) One of the referees has suggested consideration of the alternate structure i for compound **5**. Such a formulation has been discounted



i

by a comparison of the ultraviolet spectra of **5** and an authentic sample of 2-vinylquinoline. The latter exhibits its major absorption band at 245 mμ and has no fine structure in the 290–325-mμ region, whereas the former has its major absorption band at 229 mμ with five bands in the 290–325-mμ region. This observation clearly indicates the absence of the 2-vinylquinoline chromophore in compound **5** which is required for structure i. Furthermore, compound **5** would be expected to have an ultraviolet spectrum similar to that of an equimolar solution of **18a** and 1,1-diphenylethylene. That this is indeed the case has been determined experimentally.

SCHEME II



Attempts to Convert 5 into 6.—Ozonization of **5** (either isomer) gave a complex mixture of products from which 1-oxo-2-(2-acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (**14**) and 2-(2-acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-oxo-2H-pyrrolo[3,4-*b*]quinoline (**15a**) have been isolated. The desired aldehyde **6** could not be detected in the reaction mixture.

The high resolution mass spectrum of **14** established the empirical formula $C_{41}H_{43}N_3O_7$ with a molecular ion at *m/e* 689.307 (required *m/e* 689.310). This requires the addition of an oxygen atom and removal of two hydrogen atoms from the starting material **5**. The ultraviolet spectrum of **14** indicates that the new oxygen has been introduced as a carbonyl function in conjugation with the quinoline system since the band at 230 mμ in the ultraviolet spectrum of **5** has shifted to 247 mμ in the ultraviolet spectrum of **14** and the five peaks in the 290–320-mμ region in the spectrum of **5** have collapsed to a single broad band at 295 mμ in the spectrum of **14**. The nmr spectrum of **14** is very similar to that of **5** except that the two proton singlet at δ 4.25 assigned to the ring-C methylene group in the spectrum of **5** is absent in the spectrum of **14**. This suggests that the new carbonyl function is located at C-1 in **14**. The infrared spectrum of **14** also supports the placement of a new carbonyl function at C-1. The absorption at 1740 cm^{-1} is attributed to a combination of the acetate and imide I bands, the 1700-cm^{-1} band is assigned as an imide II band, and the 1625-cm^{-1} band is due to the pyrrolidine amide.[10]

The high resolution mass spectrum of the second ozonization product **15a** established the molecular formula $C_{27}H_{33}N_3O_7$ with a molecular ion at *m/e* 511.234 (required *m/e* 511.232). The empirical formula corresponds to the loss of $C_{14}H_{12}$ and the addition of one oxygen. The ultraviolet spectrum of **15a** indicates the presence of a quinoline chromophore similar to that of **14** with bands at 246 and 303 mμ. The absence of the 256-mμ shoulder which was present in the ultraviolet spectrum of **5** suggests that the $C_{14}H_{12}$ fragment lost is the diphenylethylene group. The nmr spectrum of **15a** confirms the absence of the diphenylethylene group. The infrared spectrum of **15a** suggests an imide structure similar to that of **14** with a band at 1745 cm^{-1} which is assigned as a combination of the acetate and imide I bands and a band at 1700 cm^{-1} which is the imide II band. The 1645-cm^{-1} band is due to the pyrrolidine amide.[10]

The spectral data does not allow an unequivocal choice between the 3-oxo compound **15a** and its 1-oxo isomer **16** for the structure of the $C_{27}H_{33}N_3O_7$ ozonization product. Conceivably **16** could arise from further oxidation of **14** to give the 1-oxo acid **17**[11] followed by decarboxylation[12] to give **16** (Chart I). The following observations, however, indicated that **15a** is indeed the correct structure for the $C_{27}H_{33}N_3O_7$ ozonization product.

Removal of the ketal protecting group from 2-(2-acetoxymethyl-4-carbopyrrolodinyl-4,4-diethoxybutanoyl)-1,3-dihydro-2H-pyrrolo[3,4-*b*]quinoline (**18a**) gave the corresponding ketone **18b**. Heating **18b** in acetic anhydride resulted in ring closure and aromatization to give **19**.[13] However the corresponding ketone derivative **15b** of **15a** failed to react under the same conditions due to the blockage of the reactive 3 position by the carbonyl group.

Further evidence for the assignment of **15a** as the 3-oxo isomer comes from an alternate attempt to prepare **6**. The reaction of **5** with osmium tetroxide gave 2-(2-acetoxymethyl-4-carbopyrrolodinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-(1′,2′-dihydroxy-2′,2′-diphenylethyl)-2H-pyrrolo[3,4-*b*]quinoline (**20**). When

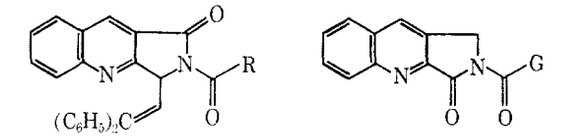
(10) (a) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley & Sons, Inc., New York, N. Y., 1958, p 221; (b) H. K. Hall, Jr., and R. Zbinden, *J. Amer. Chem. Soc.*, **80**, 6428 (1958).

(11) Carboxylic acid formation is not unusual in ozonization reactions [P. S. Bailey, *Chem. Rev.*, **58**, 925 (1958)].

(12) Pyridine-2-acetic acids decarboxylate under very mild conditions (A. R. Katritzky and J. M. Lagowski, "Heterocyclic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1960, p 98).

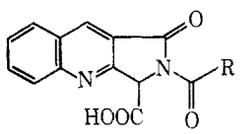
(13) M. C. Wani, J. A. Kepler, M. E. Wall, and S. G. Levine, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968, M16.

CHART I

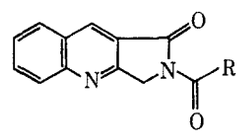


14

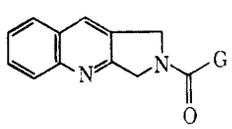
15a, G = R
b, G = R_2



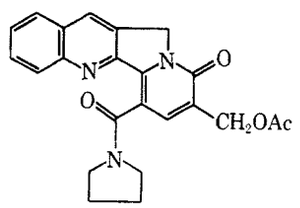
17



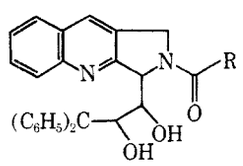
16



18a, G = R
b, G = R_2



19



20

R = $CH(CH_2OAc)CH_2C(OC_2H_5)_2CONC_4H_8$
R_2 = $CH(CH_2OAc)CH_2COCONC_4H_8$

this diol was allowed to react with an excess of periodic acid the only materials which could be detected were unreacted diol and **15a**. For **15a** to have the alternate structure **16** it would require that the 1 position of the 1,3-dihydro-2H-pyrrolo[3,4-*b*]quinoline system be susceptible to oxidation by periodic acid, but when **18a** was treated with periodic acid under the diol cleavage conditions there was no indication of oxidation at either the 1 or the 3 positions. The formation of **15a** is easily understood from the periodic acid oxidation of **20** if one assumes that the desired aldehyde **6** is formed but then undergoes further oxidation of **15a** in the presence of periodate, a reaction that is well documented for compounds which contain an active methylene group α to a carbonyl function.[14]

In a third attempt to prepare **6** the diol **20** was allowed to react with lead tetraacetate. However, the only product which could be detected was **15a**. To date attempts to convert **5** into the desired aldehyde **6** have been unsuccessful. Further studies on this transformation are presently being pursued in this laboratory. Also presently under investigation are methods of synthesis of camptothecin in which compound **18b** is an important intermediate.

(14) (a) C. A. Bunton in "Oxidation in Organic Chemistry," part A, K. B. Wiberg, Ed., Academic Press, New York, N. Y., 1965, p 388; (b) M. L. Wolfrom and S. M. Bobbitt, *J. Amer. Chem. Soc.*, **78**, 2489 (1956); (c) R. H. Cornforth, S. W. Cornforth, and G. Popjak, *Tetrahedron* **18**, 1351 (1962).

Experimental Section

General Methods.—Melting points were taken on a Kofler hot-stage microscope and are uncorrected. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer. Nmr spectra were recorded with a Varian A-60 instrument, using deuteriochloroform with tetramethylsilane as internal standard. Infrared spectra were measured with a Perkin-Elmer Model 221 instrument. Mass spectra were recorded with an Associated Electrical Industries MS-902 instrument. Microanalysis were carried out by Micro-Tech Laboratories, Skokie, Ill. Ultraviolet and high resolution mass spectra are reported only for those compounds which were homogeneous by tlc in at least two solvent systems.

2,2-Diethoxy-4-carbethoxy-5-hydroxypentanoic Acid δ-Lactone (7).—A solution of diethyl 4-formyl-2-ketoglutartate diethyl ketal[5] (146.6 g) in 800 ml of ethanol was placed in a Parr 4501 hydrogenation apparatus. Raney nickel (10 teaspoons) was added and the mixture hydrogenated at 13.6 atm with stirring for 20 hr. The solution was filtered from the catalyst and the solvent removed under vacuum. Distillation afforded 86 g of **7** which was approximately 95% pure by vpc, bp 127–134° (0.09 mm). The analytical sample was prepared by further distillation: bp 121–122° (0.05 mm); $n^{25}D$ 1.4510; ir (CH_2Cl_2) 1770 (lactone C=O), 1740 cm^{-1} (ester C=O).

Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.42; H, 7.74.

2,3-Diethoxy-4-carbethoxy-5-hydroxypentanoic Acid Pyrrolidine Amide (8).—A solution of the lactone **7** (13 g) in 20 ml of freshly distilled pyrrolidine was allowed to stand at room temperature for 18 hr and the excess pyrrolidine was removed under vacuum. Crystallization of the residue from methylene chloride–hexane gave 10.3 g of **8**: mp 95–97°; ir (CH_2Cl_2) 3420 (OH), 1720 (ester C=O), 1615 cm^{-1} (amide C=O).

Anal. Calcd for $C_{16}H_{29}NO_6$: C, 57.98; H, 8.82; N, 4.23. Found: C, 57.91; H, 8.94; N, 4.22.

2,2-Diethoxy-4-carboxy-5-hydroxypentanoic Acid Pyrrolidine Amide.—A suspension of the ester **8** (18.5 g) in 100 ml of water and 60 ml of 1 *N* sodium hydroxide was stirred at room temperature until solution was complete (~3 hr) and extracted with 50 ml of chloroform. The aqueous layer was cooled to 0° and 60 ml of 1 *N* hydrochloric acid was added slowly. The solution was immediately extracted with chloroform (five times with 150 ml); the combined chloroform layers were dried (Na_2SO_4). Removal of chloroform followed by crystallization from methylene chloride–hexane afforded 11.7 g of the desired acid: mp 118–121°; ir (CH_2Cl_2) 2750–2550 (bonded acid OH), 1725 (acid C=O), 1590 cm^{-1} (amide C=O).

Anal. Calcd for $C_{14}H_{25}NO_6$: C, 55.41; H, 8.30; N, 4.62. Found: C, 55.41; H, 8.25; N, 4.55.

5-Acetoxy-4-carboxy-2,2-diethoxypentanoic Acid Pyrrolidine Amide (3).—A solution of 10 g of 2,2-diethoxy-4-carboxy-5-hydroxypentanoic acid pyrrolidine amide in 25 ml of pyridine was added to 25 ml of acetic anhydride. After 3 hr the solution was cooled to 0° and 25 ml of methanol was added. The solvent was removed after 1 hr and the residue crystallized from methylene chloride–hexane to give 9.9 g of **3**: mp 146–148°; ir (CH_2Cl_2) 3100 (acid OH), 1720 (acid and ester C=O), 1615 cm^{-1} (amide C=O).

Anal. Calcd for $C_{16}H_{27}NO_7$: C, 55.63; H, 7.88; N, 4.06. Found: C, 55.28; H, 7.97; N, 4.03.

2-Carbethoxy-3-carbethoxyethyl-1,3-dihydro-2H-pyrrolo[3,4-*b*]quinoline (9).—A solution of 600 mg of *o*-aminobenzaldehyde[7] and 1.20 g of ethyl 1-ethoxycarbonyl-3-oxopyrrolidin-2-ylacetate[6] was dissolved in 50 ml of benzene and the solution refluxed until no more water was collected in the Dean–Stark trap. The solution was cooled to 45° and 240 mg of 50% sodium hydride in mineral oil added. The solution was allowed to stand overnight at ambient temperature. A 25-ml portion of water was added and the mixture was shaken vigorously for 10 min. The water layer was separated and adjusted to pH 2 with 1 *N* hydrochloric acid and the precipitate crystallized from ethyl acetate to afford 470 mg (31.5%) of the corresponding acid of **9**, mp 184–186°. The acid was dissolved in 100 ml of absolute ethanol saturated with hydrogen chloride and the solution allowed to stand at room temperature for 18 hr. The solvent was removed under vacuum and the residue dissolved in methylene chloride. The methylene chloride layer was washed with 10% bicarbonate solution and water and dried (Na_2SO_4). Removal of the methylene chloride afforded 450 mg of **9**: mp 114–115° (EtOAc); uv max

(EtOH) 321 mμ (ε 8140), 313 (4930), 307 (5680), 300 (3980), 293 (3980), 230 (34500).

Anal. Calcd for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 65.57; H, 6.14; N, 8.48.

2-Carbethoxy-1,3-dihydro-3-(2′,2′-diphenyl-2′-hydroxyethyl)-2H-pyrrolo[3,4-*b*]quinoline (10).—A solution of 16.4 g of **9** in 250 ml of freshly purified tetrahydrofuran was cooled to 0° and 30 ml of 3.6 *M* ethereal phenylmagnesium bromide was added under nitrogen over a 1.5-hr period. After stirring for an additional 4 hr at 0°, 2 ml more of the Grignard reagent was added and the solution stored at 4° for 18 hr. The reaction mixture was washed with water and then acidified with 6 *N* hydrochloric acid. The aqueous solution was extracted with ether, the ether layer dried (Na_2SO_4), and the solvent removed. Chromatography of the residue (alumina, activity II; 8:1 benzene–ethyl acetate) afforded 1.45 g of **9**, 3.2 g of **11** (*vide infra*), and 14.3 g of **10**: mp 167–168° (2-propanol); uv max (EtOH) 322 mμ (ε 8060), 314 (5640), 308 (6040), 300 (4330), 295 (4330), 234 sh (36300), 231 (39400), 227 sh (37500).

Anal. Calcd for $C_{28}H_{26}N_2O_3$: C, 76.67; H, 5.98; N, 6.39. Found: C, 76.82; H, 5.86; N, 6.29.

Carbamate 11.—A sample of **10** (1.54 g) was suspended in 50 ml of 95% ethanol, 2 g of potassium hydroxide added, and the solution refluxed until tlc (4:1 benzene–ethyl acetate, silica gel H) indicated that no starting material was present. The solution was diluted with water and filtered. The crude dried product weighed 1.3 g and was approximately a 9:1 mixture of **11** and **12** as estimated by nmr. Crystallization from ethanol gave pure **11**: mp 255–256°; uv max (EtOH) 322 mμ (ε 7030), 312 (5070), 306 (4690), 230 (35100).

Anal. Calcd for $C_{26}H_{20}N_2O_2$: C, 79.57; H, 5.14; N, 7.14. Found: C, 79.41; H, 5.21; N, 7.12.

1,3-Dihydro-3-(2′,2′-diphenyl-2′-hydroxyethyl)-2H-pyrrolo-[3,4-*b*]quinoline (12).—A sample of **10** (1.0 g) was suspended in 30 ml of 95% ethanol and 5 g of potassium hydroxide in 20 ml of water added. The solution was refluxed for 4 hr under nitrogen, cooled, and diluted with an equal volume of water. Filtration gave 700 mg (83%) of **12**. The analytical sample was prepared by crystallization from ethanol: mp 202–203.5°; uv max (EtOH) 319 mμ (ε 7370), 313 (5280), 306 (5670), 300 (4360), 298 (4150), 232 (32500).

Anal. Calcd for $C_{25}H_{22}N_2O$: C, 81.93; H, 6.05; N, 7.63. Found: C, 81.67; H, 6.18; N, 7.92.

2-Carbethoxy-1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo-[3,4-*b*]quinoline (13).—A solution of 1.0 g of **10** and 800 mg of *p*-toluenesulfonic acid in 50 ml of benzene was refluxed for 1 hr while collecting the water in a Dean–Stark trap. The solution was washed with sodium bicarbonate solution and water and dried (Na_2SO_4). Removal of the solvent and crystallization (2-propanol) gave 900 mg (94%) of **13**: mp 154–155°; uv max (EtOH) 321 mμ (ε 8720), 313 (5690), 307 (6450), 300 (4990), 292 (5700), 252 (22,200), 227 (48,200).

Anal. Required for $C_{28}H_{24}N_2O_2$: *m/e* 420.184. Found: *m/e* 420.183

1,3-Dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (4).—A sample of crude **11** (3.8 g) was slowly added under nitrogen with vigorous stirring to 50 ml of 48% aqueous hydrogen bromide. The reaction mixture was stirred for 3 hr and then diluted with water. The precipitated salt (4 g) was partitioned between aqueous potassium carbonate and methylene chloride. Evaporation of the dry methylene chloride layer gave the amine as a light purple foam which rapidly deteriorated upon storage. Therefore it was used without further characterization: uv max (EtOH) 320 mμ, 313, 307, 297, 265, 235; nmr ($CDCl_3$) δ 5.10 (d, 1, *J* = 9 Hz, C-3 **H**), 5.60 (S, 1, **NH**), 6.18 (d, 1, *J* = 9 Hz, olefinic **H**).

2-(2-Acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (5).—To a solution of 2.36 g of freshly prepared **4** and 2.82 g of **3** in 25 ml of purified methylene chloride was added 1.69 g of dicyclohexylcarbodiimide. The solution was allowed to stand for 15 hr and then filtered to remove the dicyclohexylurea. The filtrate was evaporated to dryness and the residue dissolved in 10 ml of ether. After the solution was allowed to stand overnight, 1.12 g of the crystalline isomer of **5** separated from it. Chromatography of the mother liquor (silica gel, 75:25 benzene–ethyl acetate) afforded an additional 850 mg of crystalline **5**. There was also obtained 1.53 g of a compound which had spectral properties nearly identical with those of **5** and which was assigned as a diastereomer of **5**. Attempts to obtain this compound in a crystalline form were not successful. The solid isomer was recrystallized from methylene chloride–methanol: mp 194–196°; uv max (EtOH) 323 mμ (ε 8410), 314 (5450), 308 (6210), 302 (4940), 295 (5450), 256 (19900), 229 (51400); ir (KBr) 1740 (ester C=O), 1640 cm^{-1} (amide C=O).

Anal. Calcd for $C_{41}H_{45}N_3O_6$: C, 72.86; H, 6.71; N, 6.22. Found (crystalline isomer): C, 72.77; H, 6.75; N, 6.22. Found (noncrystalline isomer): C, 72.43; H, 6.75; N, 6.59.

Ozonization of 5.—A solution of 160 mg (0.248 mmol) of **5** in 20 ml of 1:1 methanol–ethyl acetate was treated with ozone at Dry Ice–2-propanol bath temperature. After the calculated amount of ozone had been passed through the solution the reaction mixture was treated with an excess of trimethyl phosphite and the mixture allowed to come to room temperature overnight. The solution was taken to dryness under reduced pressure and purified by preparative tlc (silica gel HF, ethyl acetate). From this experiment was isolated 50 mg (31%) of **5**, 22 mg of an orange fluorescent material which decomposed into several products upon standing, 36 mg (25%) of **15a**, and 14 mg (7%) of **14**. The compounds **15a** and **14** were foams and could not be crystallized, but were homogeneous by tlc.

The spectral properties of **15a** follow: uv max (EtOH) 319 sh mμ (ε 5920), 303 (8890), 246 (40,300); ir (CH_2Cl_2) 1745 (ester and imide I C=O), 1645 cm^{-1} (amide C=O).

Anal. Required for $C_{27}H_{33}N_3O_7$: *m/e* 511.232. Found: *m/e* 511.234.

The spectral properties of **14** follow: uv max (EtOH) 295 mμ (ε 12800), 260 sh (2200), 247 (57,900); ir (CH_2Cl_2) 1740 (ester and imide I C=O), 1700 (imide II C=O), 1625 cm^{-1} (amide C=O).

Anal. Required for $C_{41}H_{43}N_3O_7$: *m/e* 689.310. Found: *m/e* 689.307.

2-(2-Acetoxymethyl-4-carbopyrrolidinyl-4,4-diethoxybutanoyl)-1,3-dihydro-3-(1′,2′-dihydroxy-2′,2′-diphenylethyl)-2H-pyrrolo-[3,4-*b*]quinoline (20).—A sample of **5** (100 mg) was added to a solution of 50 mg of osmium tetroxide in 5 ml of pyridine. The solution as protected from light and heated on the steam bath for 1 hr and then hydrogen sulfide was passed through the mixture. After 4 hr methylene chloride (5 ml) and ethyl acetate (5 ml) were added to the reaction mixture; the mixture was centrifuged and the organic layer decanted. Removal of the solvent gave 95.9 mg of a dark foam which was purified by preparative tlc (silica gel HF, 1:4 benzene–ethyl acetate) to give 76.4 mg of **20**, mp 171–191° (mixture of isomers). Several crystallizations from ether–hexane gave the analytical sample: mp 175–191°; uv max (EtOH) 322 mμ (ε 9480), 315 (6340), 308 (7090), 302 (5440), 295 (5190), 235 sh (51,000), 232 (55,000), 228 sh (51,900).

Anal. Calcd for $C_{41}H_{47}N_3O_8$: C, 69.37; H, 6.68; H, 5.92. Found: C, 69.06; H, 6.73; N, 5.92.

Reaction of 20 with Periodic Acid.—The diol **20** (70.0 mg) was dissolved in a mixture of 0.25 ml of water and 2 ml of dioxane and 28 mg of periodic acid was added. After 48 hr an additional 28 mg of periodic acid was added and the mixture was allowed to stand for an additional 12 hr. A small amount of sodium bisulfite was added and the solution diluted with 5 ml of water. The mixture was extracted with methylene chloride. The organic layer yielded 69.3 mg of yellow gum upon removal of the solvent. Purification of this material by preparative tlc (silica gel H, 4:1 ethyl acetate–benzene) gave 26.0 mg of starting diol and 17.2 mg of material which had properties identical with those of **15a**.

Reaction of 20 with Lead Tetraacetate.—A sample (50 mg, 0.07 mmol) of **20** was dissolved in 2 ml of benzene and 35 mg (0.08 mmol) of lead tetraacetate was added. The reaction mixture was stirred for 3 hr at ambient temperature. The reaction mixture was filtered and the solvent removed under reduced pressure. Purification of the residue by preparative tlc (silica gel HF, ethyl acetate) afforded 11 mg (22%) of **20** and 14 mg (55%) of material which had properties identical with those of **15a**.

2-(2-Acetoxymethyl-4-carbopyrrolidinyl-4-oxobutanoyl)-1,3-dihydro-3-(2′,2′-diphenylvinyl)-2H-pyrrolo[3,4-*b*]quinoline (15b).—To a solution of **15a** (10 mg) in 2 ml of tetrahydrofuran was added 2 μl of 70% perchloric acid. After 3 hr the reaction mixture was neutralized with saturated sodium bicarbonate solution and then dried (Na_2SO_4). Removal of the solvent gave 9.8 mg of slightly colored material. Purification of this material by preparative tlc (silica gel HF, 2:1 benzene–acetone) afforded 7 mg of **15b** as a foam which was homogeneous by tlc: ir (CH_2Cl_2) 1740 (acetate and imide I CO), 1720 (ketone C=O), 1700 (imide II C=O), 1640 cm^{-1} (amide C=O).

Anal. Required for $C_{28}H_{23}N_3O_6$: *m/e* 437.165. Found: *m/e* 437.163.

Attempted Cyclization of 15b.—A sample of **15b** (5 mg) was dissolved in 1 ml of acetic anhydride and the solution maintained at 100° for 18 hr. Removal of the solvent gave 4.5 mg of crude material which had spectral properties identical with those of **15b**.

Registry No.—**3**, 21902-11-4; **4**, 21902-12-5; **5**, 21902-13-6; **7**, 21902-14-7; **8**, 21902-15-8; **8**, free acid, 21902-25-0; **9**, 21902-16-9; **10**, 21902-17-0; **11**, 21902-18-1; **12**, 21902-19-2; **13**, 21902-20-5; **14**, 21902-21-6; **15a**, 21902-22-7; **15b**, 21902-23-8; **20**, 21902-24-9.

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Tumor Inhibitors. XXXIX.[1a] Active Principles of *Acnistus arborescens*. Isolation and Structural and Spectral Studies of Withaferin A and Withacnistin

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Evidence is presented for assignment of structure for two cytotoxic compounds from *Acnistus arborescens* (L.), withaferin A (**4a**), and withacnistin (**14a**). Withaferin A (**4a**) was converted to the diacetate **4b**, the ene-dione **5**, and the methanol adduct **9**, and the functional groups of each were characterized. The complete structure and stereochemistry of **4a** were established by X-ray crystallographic analysis of the monoacetate *p*-bromobenzoate **4c**. A detailed discussion of the nmr and mass spectral data is presented for the cited compounds and their derivatives. The structure of withacnistin (**14a**) was deduced, largely on the basis of ORD, nmr, and mass spectral comparison with **4a** and its derivatives, as well as by nmr solvent shift data. Conversion to the acetate **14b**, the methanol adduct **15a**, and the deacetyl methanol adduct **15c** supported the structural assignment. A small quantity of loliolide (**11**) was also isolated.

The leaves of *Acnistus arborescens* (L.) Schlecht (Solanaceae) and related species have been used for many years to treat cancerous growths.[3] In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of dried *A. arborescens* leaves[4] were evaluated and found to show significant activity *in vitro* against cells derived from human carcinoma of the nasopharnyx (KB), and *in vivo* against sarcoma 180 (SA) in mice.[5] Consequently, a systematic study aimed at the isolation of the SA-inhibitory principles was undertaken. We report herein the systematic fractionation of an active extract of *A. arborescens* and the isolation and elucidation of withaferin A, a novel steroidal lactone which shows significant inhibitory activity against the SA tumor in mice and the Walker intramuscular carcinosarcoma 256 (WM) in rats (see Table I).[6] In addition, the isolation and characterization of a companion cytotoxic[7] withanolide, withacnistin, are described.

Fractionation of an ethanol extract guided by assay against sarcoma 180 revealed that the active principle was concentrated successively in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition, the chloroform layer of a chloroform–water partition, the formamide layer of a formamide–benzene partition, and the chloroform layer of a chloroform–formamide partition. Chromatography of the chloroform-soluble material yielded withacnistin ($C_{30}H_{40}O_7$) and crystalline withaferin A ($C_{28}H_{38}O_6$). Rechromatography of mother liquors from withacnistin gave 3-ethoxy-2,3-dihydrowithacnistin and the known compound loliolide[8] (**11**).

The presence of an intense band at 214 mμ in the ultraviolet spectrum of withaferin A and a strong band at 5.92 μ in its infrared spectrum suggested the presence of α,β-unsaturated carbonyl groups. The nmr spectrum (see Table II) contained a low-field asymmetrical quartet at τ 3.05 (1 H, J_{AM} = 10.0 Hz and J_{AX} = 6.0 Hz) and doublets at τ 3.82 (1 H, J_{AM} = 10.0 Hz) and 6.25 (1 H, J_{AX} = 6.0 Hz) assignable to the AMX system **1**. Other signals were assigned to an allylic alcohol grouping, a proton on a carbon carrying an epoxide, a vinylic methyl group, and two tertiary and one secondary methyl groups.

Upon acetylation, a crystalline diacetate ($C_{32}H_{42}O_8$)

(1) (a) University of Wisconsin. Part XXXVIII: S. M. Kupchan and I. Ognyanov, *Tetrahedron Lett.*, 1709 (1969). The investigation at the University of Wisconsin was supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275). (b) Author to whom inquiries should be directed: Department of Chemistry, University of Virginia, Charlottesville, Va. 22901.

(2) University of California. This is part X in the series entitled "High Resolution Mass Spectrometry in Molecular Structure Studies." Part IX: H. K. Schnoes, D. H. Smith, A. L. Burlingame, P. W. Jeffs, and W. Döpke, *Tetrahedron*, **24**, 2825 (1968). The investigation at the University of California was supported in part by a grant from the National Aeronautics and Space Administration (NGL 05-003-003).

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(4) The plant material was collected in Costa Rica by Professor J. A. Saenz Renauld, Department of Biology, University of Costa Rica, San Jose, in Jan 1961.

(5) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(6) *Cf.* B. Shohat, S. Gitter, A. Abraham, and D. Lavie, *ibid.*, **51**, 271 (1967).

(7) Withaferin A and withacnistin showed cytotoxicity (ED_{50}) against KB cell culture at 2.8×10^{-1} and 1.7×10^{-1} μg/ml, respectively.

(8) R. Hodges and A. L. Porte, *Tetrahedron*, **20**, 1463 (1964).