98–100°; $[\alpha]^{22}D$ +48.8° (*c* 0.041, 95% EtOH); $\lambda_{max}^{KBr}$ 2840 ($OCH_3$), 1373, 1180 ($SO_3$) $cm^{-1}$; $\lambda_{max}$ in 95% ethanol $\delta$ 262 ($\epsilon$ 1200) 273 (1000), 2.45 ($ArCH_3$), 3.38 ($OCH_3$).

*Anal.* Calcd for $C_{24}H_{34}O_{10}S_2$: C, 52.73; H, 6.27; S, 11.73. Found: C, 52.67; H, 6.09; S, 11.42.

**Registry No.**—1, 21903-06-0; 1-$Ac_4$, 21903-07-1; 1,2-*O*-ethylidene-D-mannitol, 21903-08-2; 3,4,5,6-tetra-*O*-methyl-1,2-di-*O*-*p*-tolylsulfonyl-D-mannitol, 21903-09-3.

**Acknowledgments.**—The author wishes to thank Clara McGrew, Bonita Heaton, and Karen A. Jones for the microchemical analyses and W. A. Boyd for the nmr data.

# An Anomalous Stereochemical Course and a Color Phenomenon in the Addition of Anthranilic Acid to a Sugar Nitro Olefin. The Synthesis of 2,3-Diamino-2,3-dideoxy-D-mannose[1,2]

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Most additions of nucleophiles to the nitro olefin **1**, methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside, give products having a 2,3-diequatorial orientation of substituents (D-*gluco* configuration), but the 2,3-axial–equatorial orientation (D-*manno* configuration) may arise with anthranilic acid as addend. While this addend, when employed in a 2:1 molar ratio in the presence of a catalytic amount of potassium hydroxide, furnished methyl 4,6-*O*-benzylidene-2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-β-D-glucopyranoside (**2**) in 83% yield, it gave the D-*manno* isomer (**9**) of **2** in 56% yield as the sole, isolated product when the ratio of reactants was 1:1 and the catalyst was omitted. Addition to **1** of methyl anthranilate afforded only the methyl ester (**3**) of **2** and not the methyl ester (**10**) of **9**. However, either ester was obtained from its parent acid with diazomethane. The crystalline D-*manno* acid (**9**) and ester (**10**) are yellow, but are colorless in solution. Compound **2** was converted by several steps into methyl 2,3-diacetamido-2,3-dideoxy-β-D-glucopyranoside (**8**), and compound **9** was similarly converted into new, β-glycosidic derivatives of 2,3-diamino-2,3-dideoxy-D-mannose and finally into the reducing diamino sugar dihydrochloride (**14**).

Certain compounds of anthranilic acid with sugars exist in nature as bacterial metabolites. Thus, 1-(2-carboxyphenyl)amino-1-deoxy-D-ribulose has been detected in cultures of *Aerobacter aerogenes*[3] and *Escherichia coli*,[4] and 1-(2-carboxyphenyl)amino-1-deoxy-D-fructose is produced by *Salmonella typhimurium*[5] and *Neurospora crassa*.[6] These compounds, which are considered to be intermediates in the bacterial biosynthesis of tryptophan, have also been synthesized chemically.[7] To our knowledge, no sugars that carry an *o*-carboxyphenylamino group at a carbon atom other than C-1 have been described. It was therefore decided to synthesize such compounds and, at the same time, to test the scope of the synthesis of 2,3-diamino sugars recently worked out in this laboratory.[8,9] The synthesis is based on amination of 2,3-unsaturated 3-nitro glycosides followed by hydrogenation to the diamino stage.

**The Addition of Anthranilic Acid.**—Methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**), a versatile acceptor for nucleophilic addends including ammonia[8] and aliphatic amines[10] among others,[10,11] was refluxed with a 2–3 molar excess of anthranilic acid in benzene solution in the presence of a catalytic amount of potassium hydroxide. A colorless, crystalline addition product (**2**) was isolated in 83% yield (Scheme I). When an excess of anthranilic acid was maintained but the catalyst omitted, the yield of **2** was only 41%; however, an isomeric product was isolated in 22% yield, as characteristic lemon-yellow crystals. The separable amount of yellow isomer rose to 56% and the colorless isomer could no longer be isolated[12] when equimolar proportions of **1** and anthranilic acid were employed in the absence of potassium hydroxide catalyst.

The two isomers gave infrared (ir) spectra that were generally similar but differed considerably in detail (see Experimental Section). They had nearly identical melting points but widely different specific rotations. The presence of a free carboxyl group in each was demonstrated by the production of methyl esters by diazomethane. The esters differed strongly in their rotations and melting points, and the ester arising from the yellow isomer formed yellow crystals, too, whereas the other ester was colorless like its acid. It will be shown that the colorless acid was methyl 4,6-*O*-benzylidene-2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-β-D-glucopyranoside (**2**) and that the yellow acid was methyl 4,6-*O*-benzylidene-2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-β-D-mannopyranoside (**9**).

Considering the results of numerous additions of nucleophiles to **1** already reported,[8,10,11] compound **2** was the expected product. Almost all of the various addition products previously obtained from **1** had possessed the D-*gluco* configuration. A single exception[8] was a minor isomer, of then undetermined con-

(1) (a) Part XIV in a series on the reactions of nitro sugars. (b) For part XIII, see H. H. Baer and F. Rajabalee, *Can. J. Chem.*, **47**, 4086 (1969).

(2) This work was done as part of a Ph.D thesis submitted by F. K. to the University of Ottawa, 1968. It was presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968.

(3) F. W. E. Gibson, C. H. Doy, and S. B. Segall, *Nature*, **181**, 549 (1958).

(4) C. H. Doy and F. W. E. Gibson, *Biochem. J.*, **72**, 586 (1959).

(5) F. Lingens, H. Hellmann, and M. Hildiger, *Z. Naturforsch.*, **13b**, 727 (1958).

(6) F. Lingens and S. Kern, *Z. Physiol. Chem.*, **318**, 56 (1960).

(7) F. Lingens and H. Hellmann, *Ann.* **630**, 84 (1960).

(8) H. H. Baer and T. Neilson, *J. Org. Chem.*, **32**, 1068 (1967).

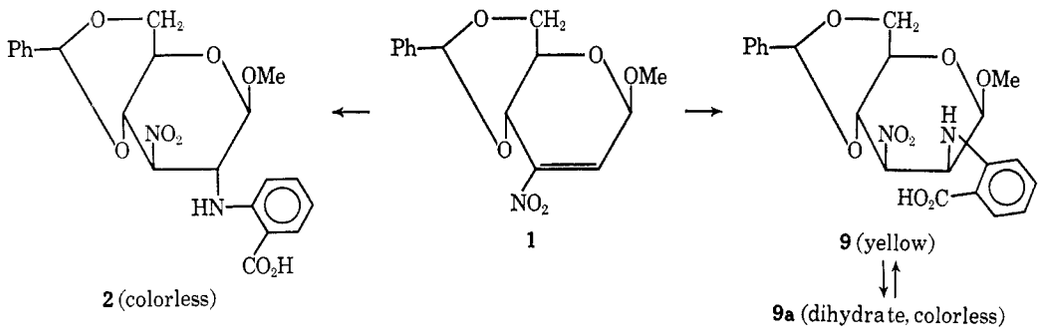
(9) H. H. Baer and K. S. Ong, *ibid.*, **34**, 560 (1969).

(10) H. H. Baer, T. Neilson, and W. Rank, *Can. J. Chem.*, **45**, 991 (1967).

(11) H. H. Baer and F. Kienzle, *J. Org. Chem.*, **32**, 3169 (1967); H. H. Baer and K. S. Ong, *Can. J. Chem.*, **46**, 2511 (1968).

(12) Whether or not a minor proportion of **2** was present cannot be stated with certainty since fractional crystallization of the mother liquor proved to be difficult.

SCHEME I

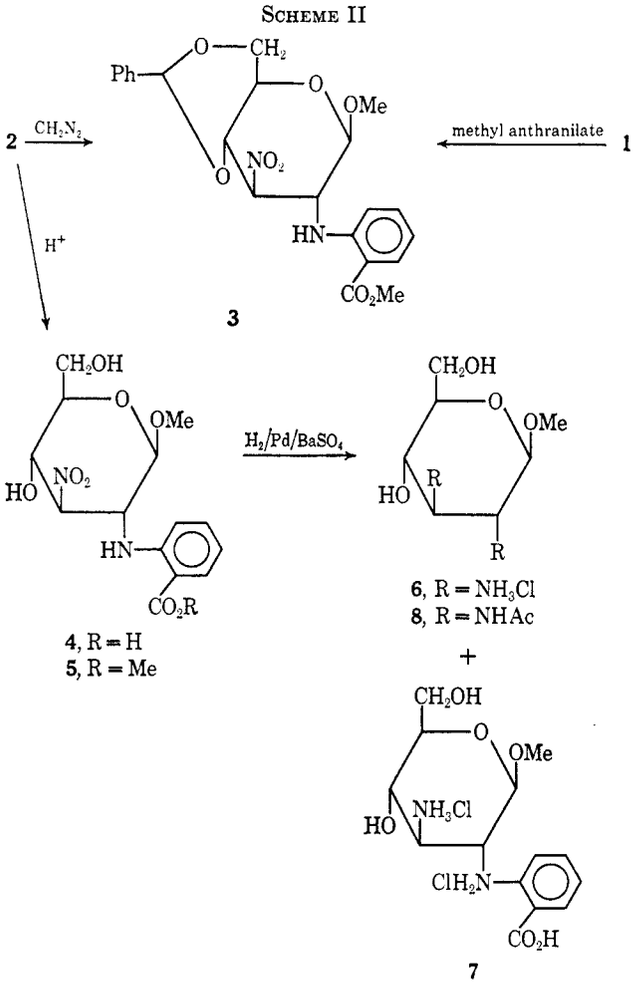



figuration, which accompanied the D-*gluco* adduct in the addition of ammonia to **1**. The D-*manno* configuration has now been assigned to that by-product (**15**). Although the formation of a D-*manno* isomer (**9**) besides the "normal" D-*gluco* derivative (**2**) was, therefore, not without precedent, it seemed surprising to observe such a directive influence of the reaction conditions upon the steric course and, in particular, to obtain **9** as the sole product, in high yield, under a specific set of conditions.[13] The formation of **9** provides a convenient route to 2,3-diamino-2,3-dideoxy-D-mannose, an amino sugar hitherto unknown.[14]

The intense, yellow color of crystalline, anhydrous **9** is presently unexplained. Solutions of **9** in chloroform, acetone, alcohols, or methyl sulfoxide are colorless. The ultraviolet (uv) spectra of "yellow" **9** and colorless **2** in methanol were very similar: **9** had two peaks at $\lambda_{max}$ 254 m$\mu$ ($\epsilon$ 11000) and 343 (5300); **2** had $\lambda_{max}$ 255 m$\mu$ ($\epsilon$ 10000) and 340 (5000). There was no absorption in the visible region. The "yellow" ester likewise was colorless in solution. Recrystallization of **9** by adding petroleum ether to a solution in cold chloroform and methyl sulfoxide gave a colorless dihydrate (**9a**). The water of crystallization apparently came from moisture present in the solvent or the atmosphere. Remarkably, however, the presence of methyl sulfoxide seemed necessary for the dihydrate to crystallize, since recrystallization of **9a** from chloroform and petroleum ether alone, or even from aqueous ethanol regenerated anhydrous **9**. The relation between **9a** and **9** was established by elemental analysis, polarimetry, mass spectroscopy (in which both gave a parent peak at *m/e* 430 and very similar fragmentation patterns), and by conversion into the same methyl ester (**10**).

**The Structure and Configuration of Compound 2.**—The reactions performed to establish the structure and configuration of compound **2** are outlined in Scheme II. The methyl ester (**3**) obtained from **2** by treatment with diazomethane was also obtained by nucleophilic addition of methyl anthranilate to **1**.[15] Debenzylidenation of **2** with aqueous acetic acid afforded methyl 2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-$\beta$-D-glucopyranoside (**4**) which gave its methyl ester (**5**) with diazomethane. Catalytic hydrogenation of **4** over the

SCHEME II




Kuhn catalyst[16] in the presence of dilute hydrochloric acid resulted in reduction of the nitro group and hydrogenolysis of the arylamino bond,[17] thus giving methyl 2,3-diamino-2,3-dideoxy-$\beta$-D-glucopyranoside dihydrochloride (**6**) in 62% yield. Methyl 3-amino-2-(2-carboxyanilino)-2,3-dideoxy-$\beta$-D-glucopyranoside dihydrochloride (**7**), which originated from incomplete hydrogenolysis, was isolated as a minor by-product (yield, 6%). *N*-Acetylation of the diamino sugar **6** produced the known[8] methyl 2,3-diacetamido-2,3-dideoxy-$\beta$-D-glucopyranoside (**8**), and it was thereby established that the new compounds **2**–**7** belonged to the D-*gluco* series.

(13) Additions of *m*- and *p*-aminobenzoic acids to **1**, and also of anthranilic acid to the $\alpha$ anomer of **1**, have furnished D-*gluco* adducts exclusively, regardless of the conditions: H. H. Baer, F. Rajabalee, and F. Kienzle, *J. Org. Chem.*, **34**, 4204 (1969).

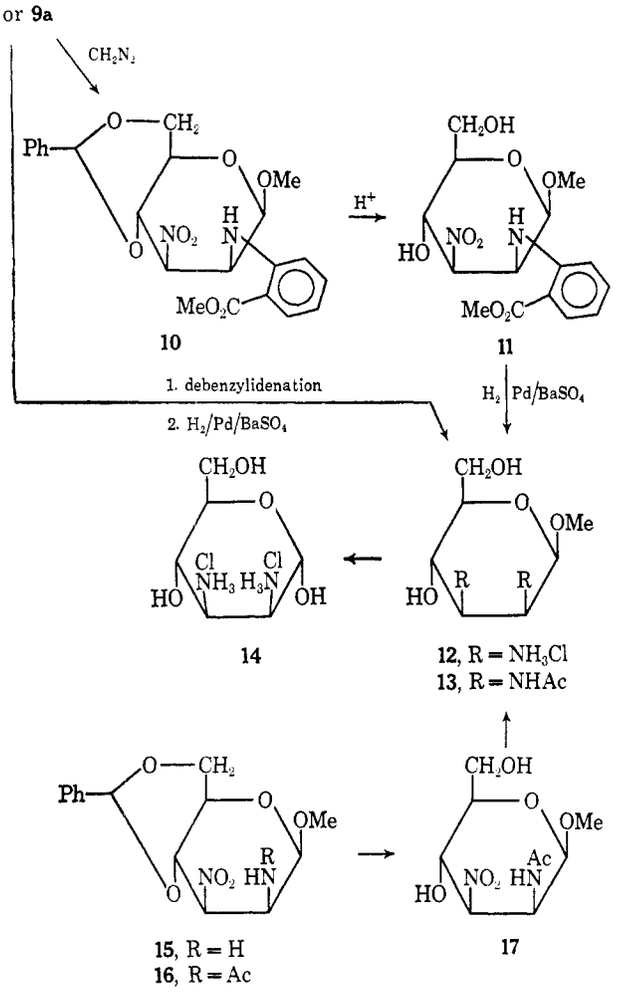
(14) Some $\alpha$-glycosidic derivatives of this sugar have been synthesized by other routes: B. R. Baker and T. L. Hullar, *J. Org. Chem.*, **30**, 4038 (1965); R. D. Guthrie and D. Murphy, *Chem. Ind.* (London), 1473 (1962).

(15) It is noteworthy that only the D-*gluco* ester (**3**) and not the isomeric D-*manno* ester (**10**) was found as a product of this addition.

(16) Brown palladium oxyhydrate on barium sulfate: R. Kuhn and H. J. Haas, *Angew. Chem.*, **67**, 785 (1955).

(17) The hydrogenolytic dearylation of arylamino sugars was first described by R. Kuhn and W. Kirschenlohr, *Ann.*, **600**, 115, 126, 135 (1956), and the mechanism was investigated by R. Kuhn and H. J. Haas, *ibid.*, **611**, 57 (1958).

SCHEME III




**The Structure and Configuration of Compound 9. Synthesis of 2,3-Diamino-2,3-dideoxy-D-mannose.**—Starting from the yellow acid **9**, the sequence of reactions depicted in Scheme III was carried out. Debenzylidenation of the methyl ester **10** with acetic acid gave methyl 2,3-dideoxy-2-[2-(methoxycarbonyl)anilino]-3-nitro-β-D-mannopyranoside (**11**) as a monohydrate. Catalytic hydrogenation of **11** (effected as described for **4**) gave methyl 2,3-diamino-2,3-dideoxy-β-D-mannopyranoside dihydrochloride (**12**). Compound **12** was also produced directly from the acid **9** by debenzylidenation followed by catalytic hydrogenation without the isolation of an intermediate. The latter route was not only shorter but afforded a purer product. However the mother liquor, according to paper chromatography, contained a fast-moving, ninhydrin-positive by-product. Although it was not isolated, there can be little doubt that it was the D-*manno* isomer of **7**, *i.e.*, a product of incomplete hydrogenolysis. *N*-Acetylation of **12** gave methyl 2,3-diacetamido-2,3-dideoxy-β-D-mannopyranoside (**13**) from which the reducing sugar, 2,3-diamino-2,3-dideoxy-D-mannose dihydrochloride (**14**), was obtained by hydrolysis with hydrochloric acid. The specific rotation of **14**, $[\alpha]_D$ +1° → −3° in water, clearly distinguished it from its known D-*gluco* isomer.[18]

None of the compounds **9**–**14** was previously known. The assignment of the D-*manno* configuration to them resulted from the following considerations and observations. Addition of a nucleophile to **1** can theoretically lead to four stereoisomers, having the D-*gluco*, D-*allo*, D-*manno*, and D-*altro* configurations. With the D-*gluco* configuration having been established for the series **2**–**8**, a choice remained among the three last-mentioned possibilities for **9**–**14**. The specific rotation of **14** was very different from that reported[19] for 2,3-diamino-2,3-dideoxy-D-allose dihydrochloride. Moreover, we were able to rule out the D-*allo* configuration experimentally as follows. It was reasoned that the D-*gluco* and D-*allo* isomers that could theoretically arise in the addition of anthranilic acid to **1** should yield a common nitronate anion in alkaline solution, and the same should be true for the D-*manno* and D-*altro* isomers, each set being a pair of C-3 epimers. The two nitronates would be expected to differ in optical rotation. Hence, alkaline solutions of **9** and **2** should have *equal* rotations if **9** was the D-*allo* isomer, but *different* rotations if **9** was the D-*manno* or the D-*altro* isomer. The experiment decided in favor of the latter alternative (Scheme IV).[20]

The final decision between the D-*manno* and D-*altro* configurations was made by comparison of the nuclear magnetic resonance (nmr) spectra of the diacetamido compounds **8** and **13**. The D-*gluco* isomer (**8**) exhibited its acetamido methyl signals at τ 8.07 and 8.09 in accordance with the diequatorial arrangement, whereas the unknown isomer (**13**) showed the corresponding signals at τ 7.96 and 8.06, which is indicative of one axial and one equatorial acetamido group.[21,22] Therefore, **13** was assigned the D-*manno* configuration.

In the synthesis of 2,3-diamino-2,3-dideoxy-D-glucose reported earlier,[8] addition of ammonia to **1**[23] had furnished a very high yield of a mixture of two stereoisomeric methyl 2-amino-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-hexopyranosides. The major isomer ($[\alpha]_D$ −49° in DMF) preponderated approximately tenfold and was shown to have the D-*gluco* configuration, while the stereochemistry of the minor isomer ($[\alpha]_D$ −96° in DMF) was not investigated at that time. The latter has now been revealed to be the D-*manno* isomer (**15**). Its briefly mentioned[8] *N*-acetyl derivative

(18) 2,3-Diamino-2,3-dideoxy-D-glucose dihydrochloride has been reported to show $[\alpha]_D$ +66.5° → +50.0° (W. Meyer zu Reckendorf, as quoted in ref 8, footnote 36) and +66° → +46.8° (ref 8).

(19) $[\alpha]_D$ +50°: W. Meyer zu Reckendorf, *Ber.*, **97**, 1275 (1964).

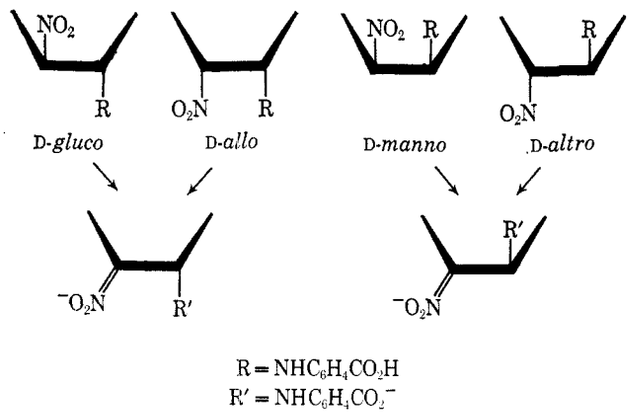
(20) The $[\alpha]_D$ values refer to freshly prepared solutions. A nearly quantitative recovery of the unchanged nitro sugars was effected by acidification shortly after taking the readings; this showed that the observed values were due to the expected salts and not to products of some secondary reaction. Secondary reactions did occur after longer times. Thus, the rotation of **9** in alkaline solution changed in the course of 20 days from −243 to −100° and then remained constant. Concurrently, a uv peak with $\lambda_{max}$ 302 mμ arose and a strong smell of benzaldehyde developed. In the light of an earlier investigation this suggested a slow, alkali-catalyzed acetal elimination leading to a 4,5-unsaturated nitronate; see H. H. Baer and F. Kienzle, *Ann.*, **695**, 192 (1966).

(21) For numerous literature references pertaining to chemical shifts of axial and equatorial acetamido groups on pyranose rings, see ref 9. Special attention is drawn to investigations on specifically deuterated derivatives, which allowed differentiation between the various types of acetamido and acetoxy groups: D. Horton, W. E. Mast, and K. D. Philips, *J. Org. Chem.*, **32**, 1471 (1967); D. Horton, J. B. Hughes, J. S. Jewell, K. D. Philips, and W. N. Turner, *ibid.*, **32**, 1073 (1967). Ample data supporting the assignments have also been collected in studies on acetamido cyclitols: F. W. Lichtenthaler and P. Emig, *Carbohyd. Res.*, **7**, 121 (1968); A. Hasegawa and H. Z. Sable, *J. Org. Chem.*, **33**, 1604 (1968).

(22) Because of insolubility of **8** and **13** in chloroform, the spectra were taken from methanol solutions (with internal tetramethylsilane standard). While most chemical shifts of carbohydrate acetamido protons reported in the literature refer to chloroform solutions, Hasegawa and Sable[21] have found that no serious deviations are introduced by methanol. The shifts observed in **8** (of proven D-*gluco* orientation) confirm this assumption.

(23) The nitro olefin **1** was used either as such or as a precursor, namely, methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside.

SCHEME IV




$R = NHC_6H_4CO_2H$
$R' = NHC_6H_4CO_2^-$

observed (*c* 0.5 in 0.1 *N* NaOH): for **2**, $[\alpha]_D$ −13.4°; for **9**, $[\alpha]_D$ −243°

(**16**), which is here characterized in more detail, was debenzylidenated to give methyl 2-acetamido-2,3-dideoxy-3-nitro-β-D-mannopyranoside (**17**), and the latter was hydrogenated catalytically under conditions effecting acetylation of the resultant amino group. The diacetamido glycoside so obtained proved to be identical with **13**.

## Experimental Section[24]

**Methyl 4,6-*O*-Benzylidene-2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-β-D-glucopyranoside (2).**—To a solution of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**)[25] (500 mg, 1.7 mmol) in dry, reagent grade benzene (50 ml) was added anthranilic acid (500 mg, 3.7 mmol) and a small chip (about 20 mg) of KOH. The mixture was boiled gently under reflux, and the progress of the reaction was monitored by tlc with ethyl acetate–petroleum ether (3:1, v/v). The fast-moving spot of **1** was completely replaced by a nonmoving spot after 5 hr. The solution was then filtered and evaporated to give a yellowish white residue which was recrystallized from warm ethanol by adding some warm water and allowing the mixture to cool slowly so as to avoid gel formation. The fine, colorless needles (610 mg, 83%) of **2**, mp 210–211°, were recrystallized once more in the same way to give pure **2** (500 mg): mp 220–221°; $[\alpha]_D$ −25.5° (*c* 1.2, acetone); ir, sharp peak at 3320 $cm^{-1}$ (NH), broad absorption extending down to the 2500-$cm^{-1}$ region ($CO_2H$), strong, single bands at 1675 (C=O) and 1555 $cm^{-1}$ ($NO_2$), and medium-strong bands at 1590, 1525, 750, and 700 $cm^{-1}$ (aromatic ring).

*Anal.* Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.60; H, 5.15; N, 6.50. Found: C, 58.46; H, 5.20; N, 6.50.

No other product could be isolated from the mother liquor.

**Methyl 4,6-*O*-Benzylidene-2-(2-carboxyanilino)-2,3-dideoxy-3-nitro-β-D-mannopyranoside (9).**—Compound **1** (300 mg, *ca.* 1 mmol) and anthranilic acid (300 mg, 2.2 mmol) were allowed to react in benzene (50 ml) as just described except that no KOH was added. A solid material (507 mg) melting at 215–218° was obtained. Three successive recrystallizations from 95% ethanol, benzene, and ethyl acetate–petroleum ether, respectively, afforded 96 mg (22%) of **9** as lemon yellow needles: mp 222° dec; $[\alpha]_D$ −173° (*c* 0.6, acetone). A mixture melting point with **2** was strongly depressed. The ir spectrum, although generally similar to that of the isomer **2**, exhibited some characteristic differences: it had a sharp peak at 3470 $cm^{-1}$ and a broad absorption consisting of several bands between 3320 and 3000 $cm^{-1}$, absorption in the 2500-$cm^{-1}$ region was less pronounced, and the C=O band was a doublet at 1700 and 1685 $cm^{-1}$ (in tetrahydrofuran solution, only a single C=O peak was given, at 1685 $cm^{-1}$); furthermore, **9** showed a strong band at 1200 $cm^{-1}$ which was absent in **2**.

*Anal.* Calcd for $C_{21}N_{22}N_2O_8$ (430.4): C, 58.60; H, 5.15; N, 6.50. Found: C, 58.72; H, 5.29; N, 6.35; mol wt, 430 (by mass spectrum).

The ethanolic mother liquor from the first recrystallization of the crude product yielded, upon addition of water, 180 mg (41%) of colorless **2**: mp 214–215° (raised to 218–219° by another recrystallization from aqueous ethanol). An ir spectrum was identical with that of **2** described above.

Compound **9** was isolated as the only product when **1** (1.0 g, 3.42 mmol) and anthranilic acid (470 mg, 3.42 mmol) were refluxed in dry benzene (100 ml) for 5 hr. Processing as just described yielded 822 mg (56%) of yellow crystals, mp 218–219° dec.

**Dihydrate 9a.**—Anhydrous **9** (100 mg) was dissolved in a mixture of chloroform (10 ml) and dimethyl sulfoxide (DMSO, 10 ml). Addition of petroleum ether to incipient turbidity and cooling (several hours at 4°) produced colorless, parallelogram-shaped plates (95 mg; 86 mg after a second recrystallization): $[\alpha]_D$ −158.5° (*c* 0.6, acetone), −150° (*c* 0.7, ethanol). The melting point was dependent on the temperature of introduction (in parentheses): 198–199 (150), 205 (160), 209 (195), and 216–217° (210°). Ir showed broad absorption in the 3500–2500-$cm^{-1}$ region, strong peaks at 1680 (C=O), 1558–1550 (doublet, $NO_2$), and 1255, 1100, and 990 $cm^{-1}$ (broad), and medium intense bands at 1605, 1589, 1518, 1302, 760, and 708 $cm^{-1}$.

*Anal.* Calcd for $C_{21}H_{22}N_2O_8 \cdot 2H_2O$ (466.4): C, 54.02; H, 5.58; N, 6.01. Found: C, 54.00; H, 5.71; N, 5.70.

The water of crystallization was lost in the mass spectrometer, and a parent peak at *m/e* 430 was found.

The dihydrate **9a** was obtained directly when **1** (480 mg) and anthranilic acid (250 mg) were refluxed for 5 hr in benzene (50 ml) containing DMSO (2 ml). Evaporation left a syrup which gave 405 mg (53%) of colorless crystals from ethyl acetate–petroleum ether. Recrystallization from the same solvents afforded 360 mg of **9a**, mp 198–199° (150°), which was identified by its ir spectrum.

When **9a** (60 mg) was dissolved in warm ethanol (8 ml) and water (2 ml) was added, yellow **9** (52 mg) crystallized on cooling to room temperature; it melted at 217–218° and was identified by its ir spectrum. Similarly, dissolution of **9a** in boiling chloroform, cooling, and addition of petroleum ether gave **9** in nearly quantitative yield.

**Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-2-[2-(methoxycarbonyl)-anilino]-3-nitro-β-D-glucopyranoside (3). A. From 2.**—The acid **2** (100 mg) in ethyl acetate (10 ml) was treated under ice cooling with ethereal diazomethane until a light yellow color persisted. Evaporation gave a white solid that was treated once more with diazomethane in the same way. The product was then recrystallized from ethyl acetate–petroleum ether to give colorless needles (74 mg): mp 225°; $[\alpha]_D$ −22.0° (*c* 0.8, acetone); ir, 3280 (NH), 1710 (C=O), and 1555 $cm^{-1}$ ($NO_2$).

*Anal.* Calcd for $C_{22}H_{24}N_2O_8$ (444.5): C, 59.45; H, 5.44; N, 6.30. Found: C, 59.34; H, 5.27; H, 6.40.

**B. From 1.**—Compound **1** (95 mg) and methyl anthranilate (49 mg, 1 molar equiv) were refluxed for 5 hr in dry benzene (25 ml). Evaporation followed by two coevaporations with toluene gave a syrup from which **3** (89 mg) crystallized with ethyl acetate–petroleum ether. It melted at 224–225°, and its ir spectrum was identical with that of **3** obtained by method A.

**Methyl 2-(2-Carboxyanilino)-2,3-dideoxy-3-nitro-β-D-glucopyranoside (4).**—Compound **2** (200 mg) was hydrolyzed with 60% acetic acid (10 ml) on a steam bath for 40 min. Removal of the solvent and repeated evaporation with added water gave a residue that was crystallized from water–ethanol (6:1) to give 112 mg of **4** as fine needles: $[\alpha]_D$ −3.0° (*c* 1, ethanol); mp 192 (160) and 204° (195°), unchanged upon recrystallization.

*Anal.* Calcd for $C_{14}H_{18}N_2O_8$ (342.3): C, 49.12; H, 5.30; N, 8.19. Found: C, 48.94; H, 5.50; N, 8.22.

**Methyl 2,3-Dideoxy-2-[2-(methoxycarbonyl)anilino]-3-nitro-β-D-glucopyranoside (5).**—Compound **4** (100 mg) was methylated with diazomethane as described for the preparation of **3** from **2**,

(24) Melting points were determined in capillaries in an electrically heated aluminum block equipped with a calibrated thermometer. The uv and visible absorptions were recorded on a Perkin-Elmer spectrophotometer, Model 202, and optical rotations were measured in a Perkin-Elmer automatic polarimeter, Model 141, at room temperature. Unless otherwise specified, ir spectra were obtained from Nujol mulls on a Beckman IR-8 instrument. The ir spectra of all new compounds were consistent with the structures assigned; individual bands are listed only where it is deemed especially helpful for purposes of identification or differentiation. All evaporations were carried out *in vacuo* at 35–40° bath temperature. Petroluem ether refers to the fraction of boiling range 30–60°. Thin layer chromatography (tlc) was performed on silica gel G (E. Merck AG, Darmstadt, Germany).

(25) H. H. Baer and T. Neilson, *Can. J. Chem.*, **43**, 840 (1965).

and **5** (91 mg) was obtained as needles: mp 161–162°; $[\alpha]_D$ +3.1° (*c* 0.6, ethanol).

*Anal.* Calcd for $C_{15}H_{20}N_2O_8$ (356.3): C, 50.56; H, 5.66; N, 7.86. Found: C, 50.43; H, 5.56; N, 7.74.

**Methyl 2,3-Diamino-2,3-dideoxy-β-D-glucopyranoside Dihydrochloride (6).**—A suspension of **4** (500 mg) in water (30 ml) and 1 *N* HCl (3 ml) was added to the Kuhn catalyst[16] (500 mg) that had been prehydrogenated in water. The mixture was shaken under hydrogen for 24 hr at ambient temperature and pressure. The solution was then filtered through a layer of Celite and evaporated with two consecutive additions of water. The brownish syrup obtained was decolorized by a treatment with activated charcoal in water, and the filtrate was evaporated with several additions of absolute ethanol. Finally **6** crystallized from absolute ethanol as platelets (213 mg, plus 12 mg from the mother liquor; yield 62%). The substance did not melt but decomposed gradually above 240°. It had $[\alpha]_D$ −41° (*c* 0.7, $H_2O$) and $R_{GN}$ 1.17.[26]

Microanalysis indicated 2% more carbon than that expected for **6**. Presumably the analytical sample contained $^1/_2$ mol of ethanol of crystallization (compare also **12**).

*Anal.* Calcd for $C_7H_{18}Cl_2N_2O_4 \cdot {}^1/_2C_2H_6O$ (288.2): C, 33.34; H, 7.18. Found: C, 33.75; H, 7.14.

**Methyl 3-Amino-2-(2-carboxyanilino)2,3-dideoxy-β-D-glucopyranoside Dihydrochloride (7).**—The ethanolic mother liquor from the crystallization of **6** was evaporated, and the residue was dissolved in water and passed through a column (1 × 15 cm) containing Dowex 1-X2 ($OH^-$) anion-exchange resin. The effluent was acidified with 1 *N* HCl, was evaporated with additions of ethanol, and yielded 12 mg of **6** (see preceding section). The column was then washed thoroughly with water (which was discarded) and eluted with 0.01 *N* HCl (250 ml). The eluate was evaporated to dryness to give an amorphous material which was treated with activated charcoal in ethanol and then brought to dryness again. The glassy substance (35 mg) failed to crystallize, but it appeared uniform in paper chromatography,[26] $R_{GN}$ 2.30. It gave ir bands indicative of hydroxyl and carboxyl groups and an aromatic ring: 3600–2500 (broad), 1670 (strong, C=O), and 750 $cm^{-1}$ (medium). It had $[\alpha]_D$ −52.5° (*c* 0.4, $H_2O$).

*Anal.* Calcd for $C_{14}H_{22}Cl_2N_2O_6$ (385.3): N, 7.27. Found: N, 7.09.

**Methyl 2,3-Diacetamido-2,3-dideoxy-β-D-glucopyranoside (8).**—A solution of the diamine dihydrochloride **6** (120 mg) in water (10 ml) and methanol (5 ml) was stirred at room temperature for 90 min with Dowex 1-X2 (carbonate form) (10 ml) and acetic anhydride (0.3 ml). Evaporation of the filtered solution afforded a partially crystalline solid that was recrystallized from acetone: colorless needles (85 mg); mp 256–258° dec, and mp 270–271° dec after another recrystallization, undepressed on admixture of **8** obtained previously;[8] $[\alpha]_D$ −103.5° (*c* 1, $H_2O$); lit.[8] $[\alpha]_D$ −105.2° (*c* 1.15, $H_2O$). The identity was confirmed by ir spectra.

**Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-2-[2-(methoxycarbonyl)-anilino]-3-nitro-β-D-mannopyranoside (10).**—This ester was obtained from **9** (700 mg) and diazomethane by the procedure given for the preparation of **3** from **2**. The product (579 mg) crystallized from ethyl acetate–petroleum ether as large, yellow prisms: $[\alpha]_D$ −188° (*c* 0.7, acetone); mp 167–168° (unchanged on recrystallization); ir, 3335 (NH), 1690 (C=O), and 1560 $cm^{-1}$ ($NO_2$). An identical product was obtained from **9a**.

*Anal.* Calcd for $C_{22}H_{24}N_2O_8$ (444.5): C, 59.45; H, 5.44; N, 6.30. Found: C, 59.47; H, 5.31; N, 6.49.

**Methyl 2,3-Dideoxy-2-[2-(methoxycarbonyl)anilino]-3-nitro-β-D-mannopyranoside (11).**—The benzylidene acetal **10** (570 mg) was hydrolyzed with 70% acetic acid (15 ml) on a steam bath for 40 min. Removal of the solvent by several coevaporations with water gave a pale yellow residue which on recrystallization from aqueous ethanol afforded **11** (405 mg, 84%), mp 82–83°. An additional recrystallization from ethyl acetate–petroleum ether gave large, yellow prisms: mp 90–91°; $[\alpha]_D$ −129° (*c* 0.7, ethanol); ir, 3600–3200 (broad, several bands), 1680 with shoulder at 1650 (C=O), and 1558 $cm^{-1}$ ($NO_2$). The crystals represented a monohydrate as indicated by microanalysis. The presence of water was confirmed by a mass spectrum taken at low ionization voltage, in which the most intense peak had *m/e* 18. The parent peak at high ionization voltage had *m/e* 356, corresponding to the molecular weight of the anhydrous compound.

*Anal.* Calcd for $C_{15}H_{20}N_2O_8 \cdot H_2O$ (374.4): C, 48.15; H, 5.89; N, 7.46. Found: C, 48.45; H, 5.92; N, 7.21.

**Methyl 2,3-Diamino-2,3-dideoxy-β-D-mannopyranoside Dihydrochloride (12). A. From 9.**—Compound **9** (200 mg) was debenzylidenated by heating in 60% acetic acid (10 ml) on a steam bath for 40 min. The syrup obtained after evaporation of the acetic acid was dissolved in water (40 ml) that contained a few drops of ethanol and 3 ml of 1 *N* HCl, and the solution was shaken under hydrogen (24 hr at ordinary temperature and pressure) in the presence of the Kuhn catalyst[16] (200 mg, prehydrogenated). The filtered solution was evaporated to give a nearly colorless syrup that could not be crystallized from a number of solvents tried. Paper chromatography[26] revealed that two ninhydrin-positive species were present, $R_{GN}$ 1.20 and 2.16. An aqueous solution of the syrup was then passed through a column (1 × 15 cm) containing Dowex 1-X2 ($OH^-$) anion-exchange resin. The effluent was acidified with 1 *N* HCl (4 ml) and evaporated to give a syrup that did not crystallize upon treatment with absolute ethanol, in contrast to the behavior of the D-*gluco* isomer (**6**). However, two evaporations with 2-propanol turned the syrup into a white powder which was isolated after trituration with dry acetone. The substance (84 mg) was homogeneous on a paper chromatogram ($R_{GN}$ 1.18) and showed $[\alpha]_D$ −45.5° (*c* 0.5, $H_2O$). Purification by treating the slightly turbid, ethanolic solution with charcoal and reprecipitating the solid with 2-propanol and acetone gave a microcrystalline material, $[\alpha]_D$ −46.5° (*c* 0.7, $H_2O$). It had no sharp melting point but sintered from about 100° (when heated at a normal rate) or decomposed with browning at about 180° (when heated very slowly). Microanalysis and nmr spectra indicated that **12** contained solvent of crystallization.[27]

*Anal.* Calcd for $C_7H_{18}Cl_2N_2O_4 \cdot {}^2/_3C_3H_8O$ (305.2): N, 9.18; Cl, 23.23. Found: N, 8.90; Cl, 23.19.

The by-product ($R_{GN}$ 2.16) could be eluted from the ion-exchange column with 1 *N* HCl, but no attempt was made to characterize this compound which was presumably the D-*manno* isomer of **7**.

**B. From 11.**—Compound **11** (255 mg) was hydrogenated as described in A for debenzylidenated **9**. The syrup obtained after removal of the solvents was twice evaporated with 2-propanol to give a white solid which was triturated with acetone and filtered (195 mg). A paper chromatogram exhibited a main spot ($R_{GN}$ 1.18) accompanied by faster moving impurities. Recrystallization from methanol–2-propanol (with charcoal treatment) gave chromatographically pure **12**, $[\alpha]_D$ −46.7° (*c* 1, $H_2O$).

**Methyl 2,3-Diacetamido-2,3-dideoxy-β-D-mannopyranoside (13).**—The diamine dihydrochloride **12** (210 mg) was *N*-acetylated as described for the preparation of **8** from **6**. The crude reaction product failed to crystallize, and tlc revealed the presence of at least three minor components besides one major product (solvent: methanol–ethanol–acetone, 1:1:2). Separation was achieved on a column (1 × 22 cm) containing 10 g of silica gel, with benzene–methanol (10:1) as eluent. Fractions (10 ml each) were inspected by tlc, and those containing only the major product were combined and evaporated. The solid residue of evaporation was boiled briefly with petroleum ether–acetone (3:1) and isolated after storage of the suspension at 4° for 24 hr. The product (57 mg), whose mobility on tlc was slightly, but distinctly, lower than that of isomeric **8**, melted over a range of 116–135° and showed $[\alpha]_D$ −108° (*c* 0.6, $H_2O$); ir, 3550–3200 (broad), 1655 amide I), 1550 (amide II), and 1075 $cm^{-1}$ (broad).

*Anal.* Calcd for $C_{11}H_{20}N_2O_6$ (276.3): C, 47.82; H, 7.30; N, 10.14. Found: C, 47.61; H, 7.36; N, 9.96.

**2,3-Diamino-2,3-dideoxy-D-mannose Dihydrochloride (14).**—The diacetamido derivative **13** (40 mg) was hydrolyzed in 5 *N* HCl (10 ml) at 100° for 2 hr. The acid was coevaporated with several portions of water, and the resulting sugar syrup was decolorized with acid-washed charcoal in water. Evaporation then gave a colorless syrup which on treatment with a small amount of ethanol–ethyl acetate (1:1) afforded white, solid **14** (30 mg):

(26) Chromatographic speed relative to D-glucosamine hydrochloride, descending on Whatman No. 1 paper in the solvent system according to F. G. Fischer and H. Dörfel, *Z. Physiol. Chem.*, **301**, 224 (1955). The spots were indicated by a ninhydrin spray.

(27) One sample showed a high-field singlet attributable to acetone, whereas a sample from a different experiment gave a high-field doublet attributable to 2-propanol (in deuterium oxide). We have recently encountered another instance of an amino sugar hydrochloride that crystallized with 2-propanol: H. H. Baer, K. Čapek, and M. C. Cook, *Can. J. Chem.*, **47**, 89 (1969).

$R_{GN}$ 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.

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

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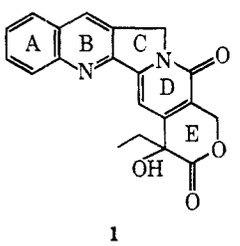
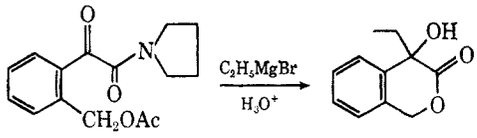
The tricyclic compound **5** containing the A,B,C ring system of camptothecin (**1**) and suitable functionality for constructing the D and E rings has been synthesized. Some of the results obtained in attempts to convert **5** into the aldehyde **6** are described.

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.