[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MAINE]

Potential Cancerocidal Agents. I. The Aromatic System of Podophyllotoxin (Part A)[1,2]

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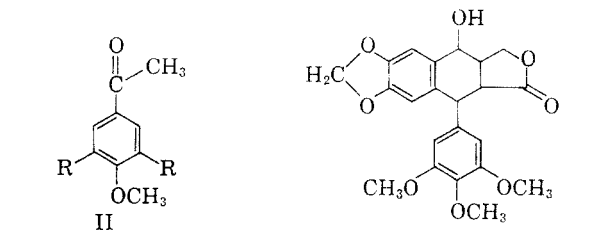
A number of tertiary amines, containing certain structural features of the tumor-damaging natural product podophyllotoxin, have been prepared for cancer chemotherapeutic studies.

The extended (1820–1942) appearance of podophyllum, the dried roots and rhizomes of certain Podophyllum species, in the U. S. Pharmacopoeia was due to its early popularity in this country as a cathartic and cholagogue.[3] Although podophyllum had received some application in early American medicine as a remedy for cancer, it was not until recently that the natural product was shown to be an effective antimitotic agent and actually useful in the treatment of condyloma acuminatum.[3]

The tumor-necrotizing action of podophyllotoxin[3,4] (I), one of the numerous components of podophyllum, is now well established; however, the unfavorable toxicity and water solubility of this substance has limited its usefulness.[5] Consequently, a variety of podophyllotoxin analogs have been prepared in attempts to provide the antitumor activity of I with a molecule presenting more desirable pharmacological properties.[6]

The present investigation was initiated in order to evaluate the effect of incorporating a dialkylaminoalkyl substituent into a molecule containing the oxygenated aromatic system of podophyllotoxin (I). Synthesis of the podophyllotoxin analog illustrated by structure IVa and several related substances appeared to offer an attractive test of this approach to useful antimitotic agents.

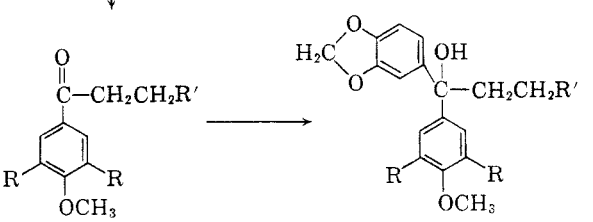
Preparation of the required compounds (IVa–e) was accomplished employing the route illustrated by the generalized formulas II→IV. Conversion of 3,4,5-trimethoxybenzoyl chloride[7] to 3,4,5-trimethoxyacetophenone (IIa), using the malonic ester procedure described by Walker and Hauser,[8] provided the necessary starting material. Condensing 3,4,5-trimethoxyacetophenone with formaldehyde and the appropriate secondary amine



II

IIa. R = OCH_3
b. R = H

I



III IV

IIIa. R = OCH_3, R′ = $N(CH_3)_2$
b. R = OCH_3, R′ = $N(CH_2CH_3)_2$
c. R = OCH_3, R′ = $N(CH_2CH_2OH)_2$
d. R = OCH_3, R′ = $N(CH_2CH_2Cl)_2$
e. R = OCH_3, R′ = N⟨⟩
f. R = OCH_3, R′ = N⟨⟩O
g. R = H, R′ = $N(CH_2CH_2OH)_2$
h. R = H, R′ = N⟨⟩O

IVa. R = OCH_3, R′ = $N(CH_3)_2$
b. R = OCH_3, R′ = $N(CH_2CH_3)_2$
c. R = OCH_3, R′ = N⟨⟩
d. R = OCH_3, R′ = N⟨⟩O
e. R = H, R′ = N⟨⟩O

(1) Abstracted in part from the Master of Science thesis submitted by D. S. Alkalay to the Graduate School, University of Maine, August 1959.

(2) This investigation was aided by Grant T-79 from the American Cancer Society.

(3) An excellent review of the history, chemistry, and pharmacology of podophyllum has been prepared by J. L. Hartwell and A. W. Schrecker, *Fortschritte der Chemie organischer Naturstoffe*, Vol. XV, L. Zechmeister, ed., Springer-Verlag, Vienna, Austria, 1958, p. 83.

(4) The chemistry of podophyllotoxin has also been reviewed by W. M. Hearon and W. S. MacGregor, *Chem. Revs.*, **55**, 957 (1955).

(5) For example, consult: G. B. Mider, *J. Nat. Cancer Inst.*, **19**, 217 (1957), and H. Seliger, *Krebsarzt*, **10**, 357 (1955).

(6) The following recent studies are pertinent to this subject: E. A. Fehnel and J. E. Stuber, *J. Org. Chem.*, **24**, 1219 (1959); M. Maturová, J. Malinský, and F. Santavý, *J. Nat. Cancer Inst.*, **22**, 297 (1959); J. Rutschmann and J. Renz, *Helv. Chim. Acta*, **42**, 890 (1959).

(7) K. H. Slotta and H. Heller, *Ber.*, **63**, 3029 (1930).

(8) H. G. Walker and C. R. Hauser, *J. Am. Chem. Soc.*, **68**, 1386 (1946).

hydrochloride led to the Mannich bases[9] represented by structures IIIa–h.

The recent work of Gensler and Stouffer[10] indicated that employing the lithium derivative of 3,4-methylenedioxybromobenzene would be superior to using the corresponding magnesium Grignard reagent to effect conversion of the intermediate Mannich bases (*e.g.* IIIa) to the tertiary alcohols (IVa–e). The desired products (IVa–e) were indeed readily prepared by allowing a tetrahydrofuran solution of 3,4-methylenedioxyphenyllithium to react at low temperature (Dry Ice–chloroform) with the respective aminoketone (III).

Interest in determining the chemotherapeutic value of several analogous compounds prompted preparation of the *N*-bis(2-hydroxyethyl) aminoketones, IIIc and IIIg, *N*-bis(2-chloroethyl)-amino-3′,4′,5′-trimethoxypropiophenone (IIId) hydrochloride, and α-[2-(*N*-morpholino)ethyl]-α-(*p*-methoxyphenyl)piperonyl alcohol (IVe).

EXPERIMENTAL[11]

β-Bis(2-hydroxyethyl)amino-4′-methoxypropiophenone (IIIg). To a solution composed of *p*-methoxyacetophenone (150 g., 1 mole), diethanolamine hydrochloride (156 g., 1.1 moles), hydrochloric acid (1 ml.) and ethanol (150 ml.) was added 60 g. of paraformaldehyde. After heating for 2 hr. at reflux, a second portion of paraformaldehyde (30 g.) was added and heating was continued an additional hour before concentrating the solution to a viscous yellow oil (460 g.) *in vacuo*. Addition of acetone (2.5 l.) precipitated the crude oily hydrochloride; weight 198 g. after drying (*in vacuo*). An aqueous solution of the hydrochloride was treated with excess sodium carbonate solution and the liquid free base which separated was isolated and added to the chloroform extract of the remaining solution. Removal of solvent from the dry (magnesium sulfate) chloroform solution gave the crude base (IIIg) as an oil which solidified to an oily yellow solid after drying (12 hr. *in vacuo*) and cooling; yield, 114 g. (42.7%), m.p. 25–32°.

A 70-g. sample of crude product (IIIg) from a similar experiment was distilled through a 12-cm. Vigreux column. The main fraction (13 g.) boiled at 126–134° (0.7–0.8 mm.) and solidified during overnight storage. Recrystallization from ethyl acetate gave 11 g. of pale yellow crystals melting at 49–52°. Two additional recrystallizations from the same solvent afforded pure colorless crystals, m.p. 58–59°, γ_{max}^{KBr} 3360 and 1658 cm.$^{-1}$

Anal. Calcd. for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 63.17; H, 7.81; N, 5.18.

β-Bis(2-hydroxyethyl)amino-3′,4′,5′-trimethoxypropiophenone (IIIc). The crude oily hydrochloride (5.3 g., 34.5%) derived from 3,4,5-trimethoxyacetophenone[8] (8.4 g., 0.04 mole), diethanolamine hydrochloride (5.25 g., 0.04 mole) and paraformaldehyde (3 g.) was converted to the free base IIIc (3.8 g., 29.2%) as described above (*cf.*, IIIg). A portion of the product (2 g.) was distilled through a 12-cm. Vigreux column and the fraction (0.5 g.) boiling at 108–115° (0.07–0.08 mm.) collected, γ_{max}^{pure} 3400 and 1670 cm.$^{-1}$

Anal. Calcd. for $C_{16}H_{25}NO_4$: C, 58.70; H, 7.70; N, 4.28. Found: C, 58.55; H, 7.52; N, 4.21.

β-Bis(2-chloroethyl)amino-3′,4′,5′-trimethoxypropiophenone (IIId) *hydrochloride*. A mixture composed of paraformaldehyde (3.6 g.), bis(2-chloroethyl)amine hydrochloride[12] (14.2 g., 0.08 mole), 3,4,5-trimethoxyacetophenone[8] (16.8 g., 0.08 mole), ethanol (12 ml.), and 1 ml. of hydrochloric acid was heated at reflux during 45 min. A second portion of ethanol (30 ml.) was added and heating continued over a 7-hr. period. The reaction mixture was allowed to cool (room temperature) overnight before collecting the colorless crystalline product; weight 4.4 g. (14%), m.p. 148–150°. Two recrystallizations from ethanol gave pure crystals melting at 151°.

Anal. Calcd. for $C_{16}H_{23}Cl_3NO_4$: C, 47.95; H, 6.04; Cl, 26.54; N, 3.50. Found: C, 47.84; H, 6.15; Cl, 26.40; N, 3.28.

β-N-Piperidino-3,4,5-trimethoxypropiophenone (IIIe). Treating an aqueous solution of *β-N*-piperidino-3,4,5-trimethoxypropiophenone hydrochloride[13] with sodium carbonate solution gave the free base (IIIe) as a colorless solid, m.p. 78–79°. Three recrystallizations from ethanol-water raised the melting point to 81.5°.

Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.42; H, 8.20; N, 4.56. Found: C, 66.22; H, 8.12; N, 4.71.

β-N-Morpholino-3,4,5-trimethoxypropiophenone (IIIf). A mixture of 3,4,5-trimethoxyacetophenone[8] (8.4 g., 0.04 mole) morpholine hydrochloride (5.0 g., 0.04 mole), paraformaldehyde (1.8 g.), ethanol (12 ml.), and 0.3 ml. of hydrochloric acid was heated to reflux. After 1 hr., an additional 1.2 g. of paraformaldehyde and 10 ml. of ethanol were added to the solution and heating continued for a total of 3 hr. The reaction mixture was concentrated to *ca.* one-half its original volume under water-aspirator vacuum and diluted with acetone (100 ml.). The resulting mixture was warmed and then allowed to cool overnight at room temperature. The principal crystalline fraction afforded 8.4 g. melting at 192–197°, while a second crop weighed 0.4 g. and melted at 201–203°; providing a total yield of 74.5%. Three recrystallizations from ethanol gave an analytical sample of *β-N-morpholino-3,4,5-trimethoxypropiophenone hydrochloride* as colorless crystals, m.p. 206–207°.

Anal. Calcd. for $C_{16}H_{24}ClNO_5$: C, 55.57; H, 7.00; Cl, 10.25; N, 4.05. Found: C, 55.80; H, 6.90; Cl, 10.29; N, 4.18.

The free base (IIIf) was obtained as a colorless solid, m.p. 95–96°, from aqueous sodium carbonate solution. Recrystallization from ethanol-water did not change the melting point.

Anal. Calcd. for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 61.97; H, 7.34; N, 4.30.

α-[2-(Dimethylamino)ethyl]-α-(3,4,5-trimethoxyphenyl)-piperonyl alcohol (IVa). To a stirred solution (under nitrogen) of 3,4-methylenedioxybromobenzene[10,14] (2.7 g., 0.0135 mole) in 15 ml. of anhydrous tetrahydrofuran, cooled to −65° (Dry Ice–chloroform), was added 6.8 ml. of ethereal 2*N* butyllithium.[15] After a 7-min. period had elapsed, an anhydrous solution of *β*-dimethylamino-3,4,5-trimethoxypropiophenone (IIIa, 3.6 g., 0.0135 mole), prepared from the corresponding hydrochloride,[13] in 15 ml. of tetrahydrofuran was added all at once. Stirring was continued an additional 20 min. before removing the cold bath and for 90 min. after cooling was discontinued. The mixture was stored overnight

(9) The Mannich reaction has been reviewed by F. F Blicke, *Org. Reactions*, **1**, 303 (1942). A more recent survey has been prepared by K. W. Merz, *Pharmazie*, **11**, 505 (1956).

(10) W. J. Gensler and J. E. Stouffer, *J. Org. Chem.*, **23**, 908 (1958).

(11) Melting points are uncorrected and were observed using the Fisher-Johns apparatus. Boiling points are also uncorrected. The infrared spectra were determined by Messrs. E. Thomas and R. Young of this laboratory. Microanalyses were provided by Dr. A. Bernhardt, Max Planck Institut, Mulheim, Germany.

(12) F. G. Mann, *J. Chem. Soc.*, 461 (1934).

(13) E. Haggett and S. Archer, *J. Am. Chem. Soc.*, **71**, 2255 (1949).

(14) K. N. Campbell, P. F. Hopper, and B. K. Campbell, *J. Org. Chem.*, **16**, 1736 (1951).

(15) H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn, and L. S. Miller, *J. Am. Chem. Soc.*, **71**, 1499 (1949).

at room temperature before removing (at 30–40°) the solvent *in vacuo*. Ammonium chloride solution was added to the residue and the crude waxy product collected, washed with water, and recrystallized from acetone; yield, 1.9 g. (38%), m.p. 110–120°. Two recrystallizations from acetone-water, followed by one from ether, gave an analytical sample as colorless crystals, m.p. 138.5–139.5°.

Anal. Calcd. for $C_{21}H_{27}NO_6$: C, 64.76; H, 6.99; N, 3.60. Found: C, 64.85; H, 6.94; N, 3.79.

α-[2-(Diethylamino)ethyl]-α-(3,4,5-trimethoxyphenyl)piperonyl alcohol (IVb). A sample of this substance was prepared employing the general procedure described above (*cf.*, IVa). The crude product from a solution of 3,4-methylenedioxybromobenzene (3.6 g., 0.018 mole) in anhydrous tetrahydrofuran (30 ml.), ethereal 2*N* butyllithium (9 ml.), and a solution of β-*N*-diethylamino-3,4,5-trimethoxypropiophenone (IIIb, 5.3 g., 0.018 mole), prepared from the hydrochloride derivative[13] in 30 ml. of anhydrous tetrahydrofuran, was recrystallized from ethanol-water; weight 3.5 g. (46.7%), m.p. 77–80°. Four recrystallizations from methanol gave a pure sample of colorless crystals melting at 108°.

Anal. Calcd. for $C_{23}H_{31}NO_6$: C, 66.16; H, 7.49; N, 3.36. Found: C, 66.02; H, 7.60; N, 3.54.

α-[2-(N-Piperidino)ethyl]-α-(3,4,5-trimethoxyphenyl) piperonyl alcohol (IVe). To a solution of 3,4-methylenedioxy bromobenzene (4.02 g., 0.02 mole) in 20 ml. of anhydrous tetrahydrofuran was added 10 ml. of 2*N* ethereal butyllithium followed by β-*N*-piperidino-3,4,5-trimethoxypropiophenone (IIIe, 6.1 g., 0.02 mole) dissolved in anhydrous tetrahydrofuran (100 ml.) as described for the preparation of IVa. Recrystallization of the crude product, 7.1 g. (79%), m.p. 80–100°, from ethanol gave a colorless crystalline analytical sample melting at 146°.

Anal. Calcd. for $C_{24}H_{31}NO_6$: C, 67.11; H, 7.28; N, 3.26. Found: C, 67.41; H, 7.42; N, 3.45.

α-[2-(N-Morpholino)ethyl]-α-(3,4,5-trimethoxyphenyl)-piperonyl alcohol (IVd). This compound was obtained by the procedure outlined for IVa, employing 3,4-methylenedioxybromobenzene (2.21 g., 0.011 mole) in anhydrous tetrahydrofuran (15 ml.), 5.5 ml. of 2*N* ethereal butyllithium and 3.43 g. (0.011 mole) of β-*N*-morpholino-3,4,5-trimethoxypropiophenone (IIIf) dissolved in 140 ml. of tetrahydrofuran. The crude product weighed 3.6 g. (76%) and melted at 156–160°. The analytical sample recrystallized from ethanol-water as colorless crystals, m.p. 161–162°, $\gamma_{max}^{CHCl_3}$ 3300–2850 cm.$^{-1}$

Anal. Calcd. for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 64.48; H, 6.92; N, 3.44.

α-[2-(N-Morpholino)ethyl]-α-(p-methoxyphenyl) piperonyl alcohol (IVe). The crude product prepared as illustrated above (*e.g.*, IVa) from ethereal 2*N* butyllithium (20 ml.), 3,4-methylenedioxybromobenzene (8.04 g., 0.04 mole) in anhydrous tetrahydrofuran (30 ml.), and β-*N*-morpholino-4-methoxypropiophenone[16] (IIIh, 10 g., 0.04 mole) in 80 ml. of anhydrous tetrahydrofuran weighed 13.3 g. (89.5%), and melted at 110–135°. Repeated recrystallization from either ethanol, ethanol-water, or benzene–petroleum ether (b.p. 60–90°) yielded pure colorless crystals, m.p. 143–143.5°, $\gamma_{max}^{CHCl_3}$ 3300–2850 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{25}NO_5$: C, 67.90; H, 6.78; N, 3.77 Found: C, 68.18; H, 6.65; N, 3.92.

ORONO, ME.

(16) T. Okuda, *Yakugaku Zasshi*, **76**, 1 (1956). *Chem. Abstr.*, **50**, 13029 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MAINE]

Potential Cancerocidal Agents. II. Synthesis of 6,7-Methylenedioxycarbostyril[1]

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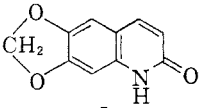
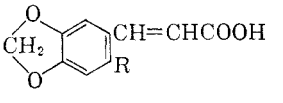
An unequivocal synthesis of 6,7-methylenedioxycarbostyril has been accomplished. Previous reports describing the preparation of this substance have been examined and reinterpreted.

During the course of a continuing search for a substance with useful antitumor activity, it was desirable to prepare 6,7-methylenedioxycarbostyril (I) for biological evaluation.[2]

Although the synthesis of 6,7-methylenedioxycarbostyril (I) had been reported by both Narang[3] and Borsche,[4] certain inconsistencies made questionable the result of each procedure. Reduction of 3,4-methylenedioxy-6-nitrocinnamic acid (IIa) with aqueous ammonia and ferrous sulfate, followed by acidification, had been claimed[3] to yield the carbostyril (I), m.p. 205°. The validity of this conclusion was doubtful in view of the experimental conditions employed and the earlier work of Perkin[5] in which the same reaction sequence had been reported to yield 3,4-methylenedioxy-6-aminocinnamic acid (IIb, brown needles, m.p. 205–207°). However, Narang[3] noted that his product apparently did not contain an amino or carboxylic acid group. Several years later, the room temperature reaction between acetic anhydride and the Schiff



I



IIa. R = NO_2
b. R = NH_2

(1) This investigation was aided by Grant T-79 from the American Cancer Society.

(2) This work constitutes part of a study concerned with the synthesis of nitrogen compounds based on certain structural features of the tumor-damaging natural product, podophyllotoxin. Consult: G. R. Pettit, and D. S. Alkalay, *J. Org. Chem.*, **25**, 1363 (1960), for the preceding paper in this series.

(3) K. S. Narang, J. N. Ray, and T. Das Sachdeva, *J. Indian Chem. Soc.*, **13**, 260 (1936).

(4) W. Borsche and W. Ried, *Ann.*, **554**, 269 (1943).

(5) F. M. Perkin, *J. Chem. Soc.*, **59**, 150 (1891).