

Potential Anticancer Compounds with Structural Relation to Podophyllotoxin

Synthesis of 5-(4',5',6'-Trimethoxyphenyl)-bis-lactone (3-Hydroxymethyl-4-carboxy and 1-Hydroxymethyl-6-carboxy)-3,6-cyclohexadiene and Related Compounds

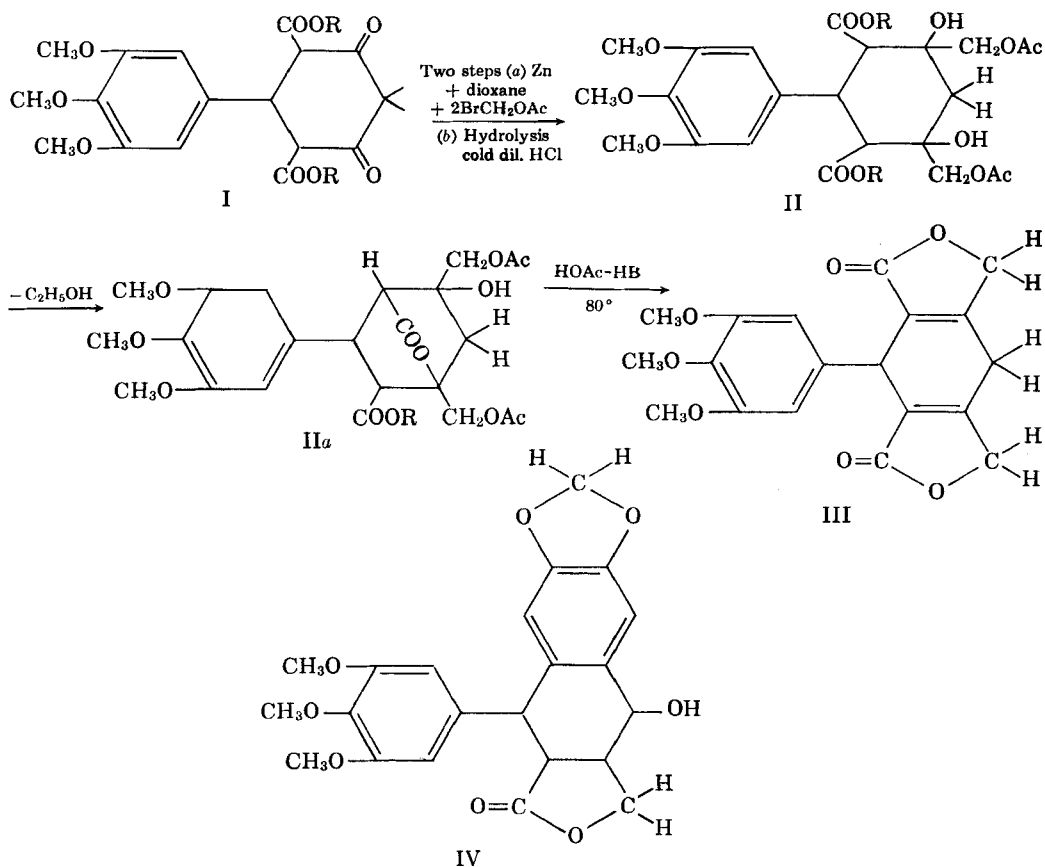
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Compounds with structural relation to podophyllotoxin and the peltatins were synthesized as potential anticancer reagents.

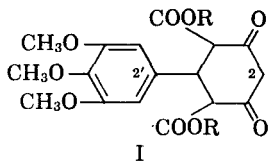
It is known that from the rhizomes of *Podophyllum peltatum*, an extract, podophyllin, has been obtained which has anticancer properties and causes hemorrhage and necrosis to the cells of sarcoma 37. The active principles of podophyllin—namely, podophyllotoxin, α and β

peltatins, and demethylpodophyllotoxin—were isolated in crystalline form, and their structure and properties have been studied extensively (1-7).

The object of this research is to synthesize compounds with structural relation to podophyllotoxin and peltatins. It is hoped that the new substances may have anticancer properties but not the toxicity of podophyllotoxin.



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R = C₂H₅

DISCUSSION

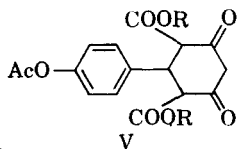
In a previous publication (8), the preparation of 5-(4',5',6'-trimethoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 (I) was reported. It was stated that compound I was to be used as an intermediate for the synthesis of substances having structural relation to anticancer agents or to other substances of physiological importance.

For the synthesis of I, 3,4,5-trimethoxybenzaldehyde was used as the starting material. This was prepared previously (8) from 3,4,5-trimethoxybenzoylchloride using the Sonn-Muller reaction. In this work, a more direct method was used with good results. The 3,4,5-trimethoxybenzoylchloride was reduced to the corresponding aldehyde by lithium aluminum tri-tertiarybutoxy hydride (9, 10). The sequence of synthesis of I and the procedures used have been described (8). Compound I was reacted with bromomethyl acetate and Zn. (The bromomethyl acetate is not an α carbon halogen ester, so it is not a Reformatsky type of reagent.) This new type of reaction was used by the senior author previously.

A mixture of compound I, bromomethyl acetate, and dry dioxane was added slowly to a flask containing purified Zn and dry dioxane. The organo-zinc complex formed was hydrolyzed; the product was extracted with toluene. The solvent was evaporated, and the gelatinous residue was dissolved in methyl alcohol. Part of the solvent was distilled off. After cooling, white flakes crystallized out. The analysis corresponds to compound IIa.

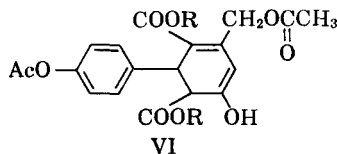
Compound IIa with the functional groups, —COOR and —CH₂OAc adjacent carbons, can be converted to a lactone according to the procedure used by Elderfield (11). The analytical results of the product correspond to the molecular empirical formula, C₁₉H₁₈O₇. From the method of synthesis, we are led to believe that it corresponds to III. By resonance, other structures are possible. III has structural similarities to podophyllotoxin (IV) and to the peltatins, which have anticancer properties.

For preliminary work to the preparation of compound III, 5-(*p*-acetoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 (V) was used as the starting material instead of the 5-(4',5',6'-trimethoxyphenyl)-4,6-dicarbethoxycyclohexanedione-1,3 (I). The reason for choosing V for the preliminary work is that V is easier to obtain than I. The method of preparation of V has been described (12).

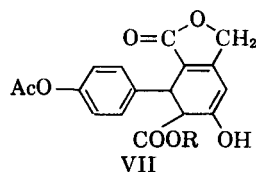


Compound V was treated with bromomethylacetate and Zn in the molar proportion of 1:1:1,

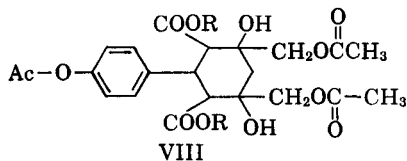
respectively. The procedure is described under *Experimental*. A compound was obtained, m.p. 128–130°, the analysis of which corresponds to the molecular empirical formula, C₂₃H₂₆O₈, which may be represented by VI.



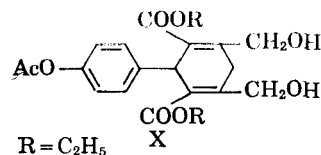
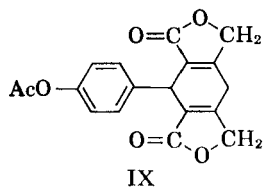
When VI was dissolved in glacial acetic acid, saturated with HBr at low temperature, then heated under reflux at 80° for about 2 hr. using the method of Elderfield (11), a compound, m.p. 150–152°, with the molecular empirical formula, C₁₉H₁₈O₇, was obtained (VII).



In another experiment, where the molar proportion of compound V to bromomethylacetate and to Zn was 1:2:2, respectively, and the procedure similar to that used for the preparation of VII, a compound, m.p. 180–186°, with the molecular empirical formula, C₂₆H₃₄O₁₂, was obtained (VIII).

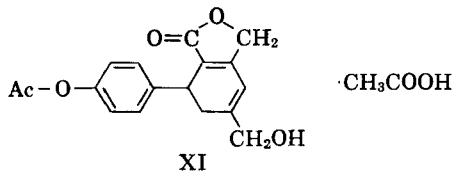


In the next step, compound VIII was dissolved in glacial acetic acid (11) saturated with HBr, and heated at 80° under reflux for 5 hr. The product of this reaction was not the expected (IX), but a compound, m.p. 198–201°, with the molecular empirical formula, C₂₂H₂₆O₈, which may be an intermediate, such as X.



In an attempt to convert the compound with the assumed formula (X) to IX, it was dissolved in glacial acetic acid saturated with HBr and then heated at 80° under reflux for 5 hr. A compound

was isolated, m.p. 170–173°, with the molecular empirical formula, $C_{19}H_{20}O_7$. It was recrystallized from three different solvents—namely, acetic acid, methanol, and ethyl acetate—and in each case the recrystallized product was analyzed for per cent carbon and per cent hydrogen; the three analyses check each other. The analytical results suggest that in the process CO_2 was lost, and the product may be represented by XI.



EXPERIMENTAL

3,4,5-Trimethoxybenzaldehyde.—3,4,5-Trimethoxybenzoylchloride, 92.4 Gm. (0.401 mole), was dissolved in 200 ml. of diglyme and placed in a three-necked 1-L. flask fitted with a stirrer, separator, and nitrogen inlet. The flask was flushed with dry nitrogen and cooled to -78° by immersing it in a cooling bath of dry ice and trichloroethylene. A 50.8-Gm. quantity of $Li-Al(t-OBu)_2H$ was dissolved in sufficient diglyme to make a solution of 200 ml. This solution was placed in the separator and added dropwise while stirring over a period of 1 hr. into the three-necked flask. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature; then it was refluxed for 2 hr. and poured into a mixture of 500 Gm. of crushed ice and 100 ml. of 10% H_2SO_4 . The pure white precipitate was filtered, pressed dry, and extracted several times with 95% ethanol. Distillation of the solvent gave 62 Gm. of crude aldehyde, m.p. 65–74°. Recrystallization from hot ethanol-water (1:1) gave 52.82 Gm. of white flakes, m.p. 74.5–75°; yield, 62%; analysis by the Detroit Testing Laboratory, 1 μ ., May 31, 1963.

Anal.—Calcd. for $C_{10}H_{12}O_4$: C, 61.22; H, 6.12. Found: C, 61.03; H, 6.05.

5-(4',5',6'-Trimethoxyphenyl)-1-hydroxy-1,3-diacetoxymethyl(3-hydroxy-6-carboxy cross lactone)-4-carbethoxycyclohexane (IIa).—The zinc used was cleaned by washing with dilute hydrochloric acid, distilled water, absolute alcohol, and ether; then it was placed in an oven at 100° to dry any moisture that may have condensed upon the evaporation of the ether. A 1.2-Gm. quantity of zinc was placed in a dry flask with 5 ml. of dioxane (previously dried over sodium). A 0.60-Gm. (0.0014 mole) quantity of 5-(4',5',6'-trimethoxyphenyl)-4,6-dicarbethoxycyclohexanone-1,3 (1, 12) was dissolved in 30 ml. of dioxane to which 0.434 Gm. (0.0028 mole; 0.36 ml.) of bromomethyl acetate was added and placed in a separator. A 0.4-ml. quantity of the solution was added slowly to the zinc, and the flask was warmed gently until the reaction started. Then the mixture was gently stirred, and the rest of the solution was added at such a rate that moderate refluxing occurred for about 1 hr. The light yellow liquid was further refluxed on an oil bath for 30 min. The flask was allowed to cool, then placed in an ice bath. Ten milliliters of 10% hydrochloric acid was added with stir-

ring. Fifty milliliters of toluene was added, and the mixture was transferred to a separator. The water layer was removed, and the toluene layer was washed twice with 10 ml. of 5% HCl, then with 25 ml. of water. The acid solutions were combined and extracted with toluene. The combined toluene portions were dried for 24 hr. over anhydrous sodium sulfate. The sodium sulfate was filtered off, and the toluene was removed by distillation under reduced pressure. The yellow gelatinous residue was recrystallized from methanol. A 0.52-Gm. quantity of white flakes was obtained, 40% of the theoretical yield, m.p. 168–170°.

Anal.—Calcd. for $C_{27}H_{38}O_{13} - C_2H_5OH$: C, 57.25; H, 6.10. Found: C, 57.39; H, 5.73.

5-(4',5',6'-Trimethoxyphenyl)-bis-lactone of [3-Hydroxymethyl-4-carboxy and 1-Hydroxymethyl-6-carboxy]-cyclohexadiene-3,6 (III).—The unsaturated lactone rings of III were synthesized according to a method described by Elderfield (11). Glacial acetic acid was saturated with dry HBr at low temperature. To the mixture of HBr-acetic acid, 0.42 Gm. of compound IIa dissolved in 5 ml. of glacial acetic acid was added and further saturated with dry HBr. The ice bath then was removed; the solution was heated under a reflux condenser at a temperature of 80° . After 0.5 hr., a low-boiling fraction was removed by distillation, and the remainder was further kept at 80° under reflux for 5 hr. The mixture was poured into ice water. The precipitate formed was separated and washed with 10 ml. of 5% HCl, then with two 25-ml. portions of water. The product was dissolved in ethyl acetate. After drying, the solvent was removed under reduced pressure using a water bath. The residue was dissolved in methyl alcohol and precipitated with water. The flocculent light tan crystals were filtered by suction; yield, 0.12 Gm. (46%), m.p. 180–182° (III).

Anal.—Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.62; H, 5.18.

5-(p-Acetoxyphenyl)-1-acetoxymethyl-3-hydroxy-4,6-dicarbethoxycyclohexadiene-2,6 (VI).—The zinc was purified by washing with dilute hydrochloric acid, distilled water, absolute alcohol, and ether. It was dried in an oven at 100° .

In a clean dry 250-ml. three-necked flask equipped with a magnetic stirrer, a separator, and a reflux condenser, the upper end of which was protected by a calcium chloride tube, was placed 3.8 Gm. of purified zinc with 15 ml. of dried dioxane. A solution of 1.03 Gm. (0.02 mole, 2.54 ml.) of bromomethylacetate and 7.8 Gm. (0.02 mole) of compound V in 50 ml. of dioxane was placed in the separator. About 5 ml. of this solution was added to the zinc, and the flask was warmed gently until reaction started. The mixture then was stirred, and the rest of the solution was added to the zinc at such a rate that gentle refluxing occurred for about 1 hr. The greenish-yellow liquid was refluxed on an oil bath for an additional 0.5 hr. The flask then was cooled in an ice bath and the contents poured into 75 ml. of ice-cold 10% hydrochloric acid with continuous stirring. One hundred milliliters of dried toluene was added, and the mixture was transferred to a separator. The acid layer was removed and the toluene layer washed twice with 25 ml. of 5% hydrochloric acid and with 25 ml. of water. The combined acid solutions were washed with two

25-ml. portions of toluene. The dioxane-toluene extracts then were combined and dried for 24 hr. over anhydrous sodium sulfate.

After filtration, the solvent was removed by distillation at atmospheric pressure on a steam bath, and the residue was distilled under reduced pressure to dryness. The orange-yellow residue was removed from the claisen flask with methanol and precipitated with distilled water. The white flakes melted at 128–130°. The yield was 5.3 Gm. (49%).

Anal.—Calcd. for $C_{23}H_{26}O_9$: C, 61.87; H, 5.87. Found: C, 61.59; H, 5.83.

5-(*p*-Acetoxyphenyl)-3-hydroxy-4-carbomethoxy-lactone of (6-Carboxy-1-hydroxymethyl)-cyclohexadiene-2,6 (VII).—A three-necked 100-ml. flask was equipped with a condenser, an HBr inlet, and a magnetic stirrer. Twenty-five milliliters of glacial acetic acid was placed in the flask and saturated with dry HBr at 0°. To this mixture 5.0 Gm. of the methyl bromoacetate product dissolved in 20 ml. of glacial acetic acid was added and further saturated with dry HBr for approximately 20 min. The ice bath was then removed, and the solution was heated to reflux at 80°. At the end of 0.5 hr., a low-boiling fraction was removed by distillation, and the residue was further refluxed at 80° for a total of 5 hr.

Then the contents were poured onto a mixture of 150 Gm. of crushed ice and 150 ml. of 5% HCl. The product was extracted with ethyl acetate and dried over anhydrous sodium sulfate for 24 hr. The ethyl acetate extract, after washing free from acid, drying, and removing the solvent by distillation under reduced pressure on a steam bath, left a tan residue. Recrystallization from methanol gave 21 Gm. of light tan crystals (55%), m.p. 150–152°.

Anal.—Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.80; H, 5.30.

5-(*p*-Acetoxyphenyl)-1,3-dihydroxy-1,3-diacetoxymethyl-4,6-dicarboxycyclohexane (VIII).—The procedure of this preparation was similar to that of the preparation of compound VI, except that the molar proportion of V to bromomethylacetate to Zn was as 1:2:2. After the mixture was prepared, reacted, and treated, it was allowed to stand overnight, wherein large white needle-like crystals formed in the greenish liquid. Upon removal of a few crystals, they proved to be hygroscopic. The dioxane-toluene mixture then was distilled under reduced pressure to dryness. The yellow residue was removed from the claisen flask with methanol and precipitated with distilled water. The light tan flocculent crystals were filtered with suction and oven-dried. A dried portion melted at 180–186°; the yield was 27 Gm.

Anal.—Calcd. for $C_{26}H_{34}O_{12}$: C, 57.99; H, 6.31. Found: C, 57.75; H, 6.08.

Attempt to Prepare 5-(*p*-Acetoxyphenyl)-bis-lactone of 3-Hydroxymethyl-4-carboxy and 1-Hydroxymethyl-6-carboxy-cyclohexadiene-3,6 (IX).—A three-necked 250-ml. flask was equipped with a condenser, hydrogen bromide inlet, and

magnetic stirrer. Seventy-five milliliters of glacial acetic acid was placed in the flask and saturated with dry HBr at 0°. To this mixture, 25 Gm. of compound VIII dissolved in 100 ml. of glacial acetic acid was added and further saturated with HBr for approximately 20 min. The ice bath was removed and the solution heated to reflux at 80°. At the end of 0.5 hr., a low-boiling fraction was removed by distillation, and the residue was refluxed further at 80° for a total of 5 hr. The contents then were poured onto a mixture of 250 Gm. of crushed ice and 250 ml. of 5% HCl. (The product was insoluble in ice water.) The product was extracted with ethyl acetate and dried over anhydrous sodium sulfate for 24 hr. The ethyl acetate extract, after washing free of acid, drying, and removing the solvent by distillation under reduced pressure on a steam bath, left an orange-brown residue. Recrystallization from methanol gave 20 Gm. of light orange crystals, m.p. 198–201°.

Anal.—Calcd. for $C_{19}H_{14}O_6$: C, 66.25; H, 4.29. Found: C, 63.59; H, 5.99.

The analytical data indicated that the desired adduct (XII) had not been obtained. However, a possible intermediate, 5-(*p*-acetoxyphenyl)-1,3-di-(hydroxymethyl)-4,6-dicarboxycyclohexadiene-3,6 (XIII), may have resulted, indicating that the reaction was not complete.

Anal.—Calcd. for $C_{22}H_{26}O_8$: C, 63.13; H, 6.22. Found: C, 63.59; H, 5.99.

Attempt to Convert X to IX.—Compound X was dissolved in glacial acetic acid and the solution saturated with HBr at low temperature, then heated at 80° under reflux for 5 hr. The procedure was a repetition of that above. The new product melted at 170–173°. The analytical results do not agree with IX but indicate a molecular empirical formula $C_{19}H_{20}O_7$ (XI). The product was recrystallized from three different solvents—namely, acetic acid, methanol, and ethyl acetate—and the recrystallized product was analyzed in each case.

Anal.—Calcd. for $C_{19}H_{20}O_7$: C, 63.32; H, 5.59. Found: C, 63.04, 63.37, 63.22; av. C, 63.22; H, 5.94, 5.78, 5.41; av. H, 5.71.

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