Investigation of the Antitumor Activity of Podocarpic Acid Derivatives

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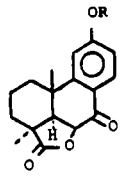
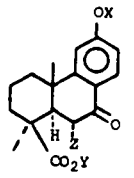
Abstract □ As a class, octahydrophenanthrene lactones, podolactones, and related podocarpic acid derivatives have been reported to possess a wide variety of biological activities, including antileukemic activity, inhibition of plant cell growth, and hormonal and anti-inflammatory properties. In the present study, a series of synthetic intermediates derived from podocarpic acid have been prepared and evaluated with respect to their ability to inhibit human epidermoid carcinoma of the nasopharynx *in vitro*. The significant cytotoxicity demonstrated by methyl 6α-bromo-7-oxo-*O*-methylpodocarpate (50% inhibition of cells at 8.85×10^{-9} M) was markedly higher than that of the other derivatives examined. Further evaluation against L1210 and P388 lymphoid leukemias in mice failed to demonstrate significant antitumor activity.

Keyphrases □ Podocarpic acid derivatives—synthesis, antitumor activity, *in vitro* human epidermoid carcinoma (nasopharynx) screen, L1210 and P388 screens in mice □ Antineoplastic agents—potential, podocarpic acid derivatives, synthesis, *in vitro* human epidermoid carcinoma (nasopharynx) screen, L1210 and P388 screens in mice

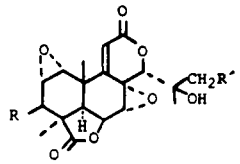
Octahydrophenanthrene lactones (I) and related podocarpic acid derivatives (II) have been reported to possess hormonal and anti-inflammatory properties (1). Other similar podolactones have been shown to inhibit the expansion and division of plant cells (III) (2–4) and to have antileukemic activity (IV) (5). In view of their documented biological properties it appeared worthwhile to test a series of synthetic intermediates derived from podocarpic acid for antitumor activity. This report describes the preparation of these derivatives and the results obtained in biological tests against tumors.



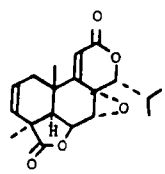
Ia, R=H
Ib, R=CH_3
Ic, R=CH_2CH_3



II a, X=Y=CH_3, Z=H
II b, X=Y=CH_3, Z=Br
II c, X=CH_3, Y=Z=H
II d, X=CH_2CH_3, Y=CH_3, Z=H



III a, R=H, R'=OH
III b, R=CH_3, R'=OH
III c, R=H, R'=$SOCH_3$



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RESULTS AND DISCUSSION

Preparation of all derivatives shown in Scheme I proceeded *via* methyl *O*-methyl-7-ketopodocarpate (VI), which was synthesized from podocarpic acid by methylation with dimethyl sulfate and subsequent oxidation using the approach described by Bible and Grove (1) in the synthesis of bromoketone (IIb).

Compound VII was prepared from VI by reduction of the 7-keto group to the corresponding alcohol with sodium borohydride. The resulting epimeric alcohol mixture (the 7α-hydroxyl derivative would be expected to be present in greater abundance) was efficiently dehydrated by *p*-toluenesulfonic acid in refluxing dihydropyran. The synthesis of epoxide VIII was completed by treatment of VII with *m*-chloroperbenzoic acid in ether. The α-configuration of the epoxide in VIII is inferred from X-ray crystallographic studies (6) of IIb and related 6α-bromo-7-oxo-diterpenoids (7). These studies indicate significant intramolecular steric hindrance in that region of the molecule resulting from 1,3-diaxial interactions of the angular methyl group at C-10 and the axial carbomethoxy group at C-4. Both of these groups reside on the β face of the molecule and would mediate an α-approach of the peracid.

Bromoketone IIb was prepared by an adapted procedure derived from the work of Bible and Grove (1). Compounds IX and X were prepared from bromoketone IIb by treatment with *N*-phenylbenzamidine and 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU), respectively, utilizing procedures reported previously (8, 9).

The activity of the freshly prepared synthetic intermediates (Scheme I) against human epidermoid carcinoma of the nasopharynx is shown in Table I. The *in vitro* activity of bromoketone IIb in the 9KB test system was markedly higher than that of VII–X. Since the cytotoxicity demonstrated by IIb was sufficient for confirmation as an active compound at the National Cancer Institute, these compounds were tested in the LE and PS tumor systems (Table II). All derivatives were inactive in both the L1210 and PS tumor systems.

EXPERIMENTAL[1]

Methyl *O*-Methyl-7-ketopodocarpate (VI)—To crude podocarpic acid (V, 500 g) was added ice (500 g), methanol (500 mL), and sodium hydroxide (240 g). This mixture was stirred for 2 h until a dark-brown color persisted, indicative of the formation of the phenoxy anion. The solution was cooled to 15°C and dimethyl sulfate (425 mL, 566.5 g, 4.5 mol) was added dropwise, with stirring, over a period of 1.5 h. The reaction was completed, as evidenced by solidification of the solution. A light-brown solid was obtained by filtration after the addition of 1 L of water. This solid was stirred with 1 L of water and removed by filtration. Recrystallization from acetone yielded 460.1 g of methyl *O*-methylpodocarpate as white crystals, mp 127.0–128.5°C [lit. (10) mp 128°C]. IR(KBr): 1725, 1500, and 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.10 (s, 3, C—CH_3), 1.32 (s, 3, C—CH_3), 3.70 (s, 3, O—CH_3), 3.80 (s, 3, O—CH_3), 6.71 (m, 2, ArH), and 7.72 ppm (d, 1, J = 7.5 Hz, ArH).

Methyl *O*-methylpodocarpate (100 g, 0.331 mol) was dissolved in 1 L of acetic acid. Chromium trioxide (94 g, 0.93 mol) was dissolved in an 800-mL acetic acid–200-mL water solution, which was then added to the ester solution at room temperature with stirring. After allowing the mixture to stand overnight at room temperature, it was poured into 6 L of saturated aqueous sodium chloride solution. After allowing this solution to stand overnight, the precipitate was removed by filtration and washed with water to yield 74 g (71%) of ketone VI as yellow crystals, mp 121–123°C [lit. mp 121–123°C (11) and 122–124°C (1)]. IR (KBr): 1725 and 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.98 (s, 3, C—CH_3), 1.20 (s, 3, C—CH_3), 3.68 (s, 3, O—CH_3), 3.86 (s, 3, O—CH_3), 6.66 (m, 2, ArH), and 7.83 ppm (d, 1, J = 8 Hz, ArH).

Compound VII—Methyl *O*-methyl-7-ketopodocarpate (VI, 10 g, 31.6 mmol) was dissolved in tetrahydrofuran (14 mL) and 95% ethanol (20 mL). After dissolving sodium borohydride (2 g) in water (8 mL) and 95% ethanol (3 mL), this solution was added slowly to the ketoester solution. The resulting solution was stirred for 24 h at room temperature, after which the precipitate

[1] IR spectra were obtained using a Perkin-Elmer Model 137 G or a Perkin-Elmer Model 580 spectrophotometer. NMR were obtained on a Jeolco Minimar or a Varian EM-390 spectrometer using $CDCl_3$ as the solvent; tetramethylsilane was used as an internal standard. Mass spectra were obtained using a Perkin-Elmer model 270 mass spectrometer. GC was performed using a Hewlett-Packard Model 402 gas chromatograph with a hydrogen flame detector. A glass column packed with 3% SE-30 on 100–120 mesh GCQ at a column temperature at 270°C with a nitrogen flow rate of 35 mL/min was used for all GC analyses unless otherwise stated. Melting points were obtained on a Fisher-Jones apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

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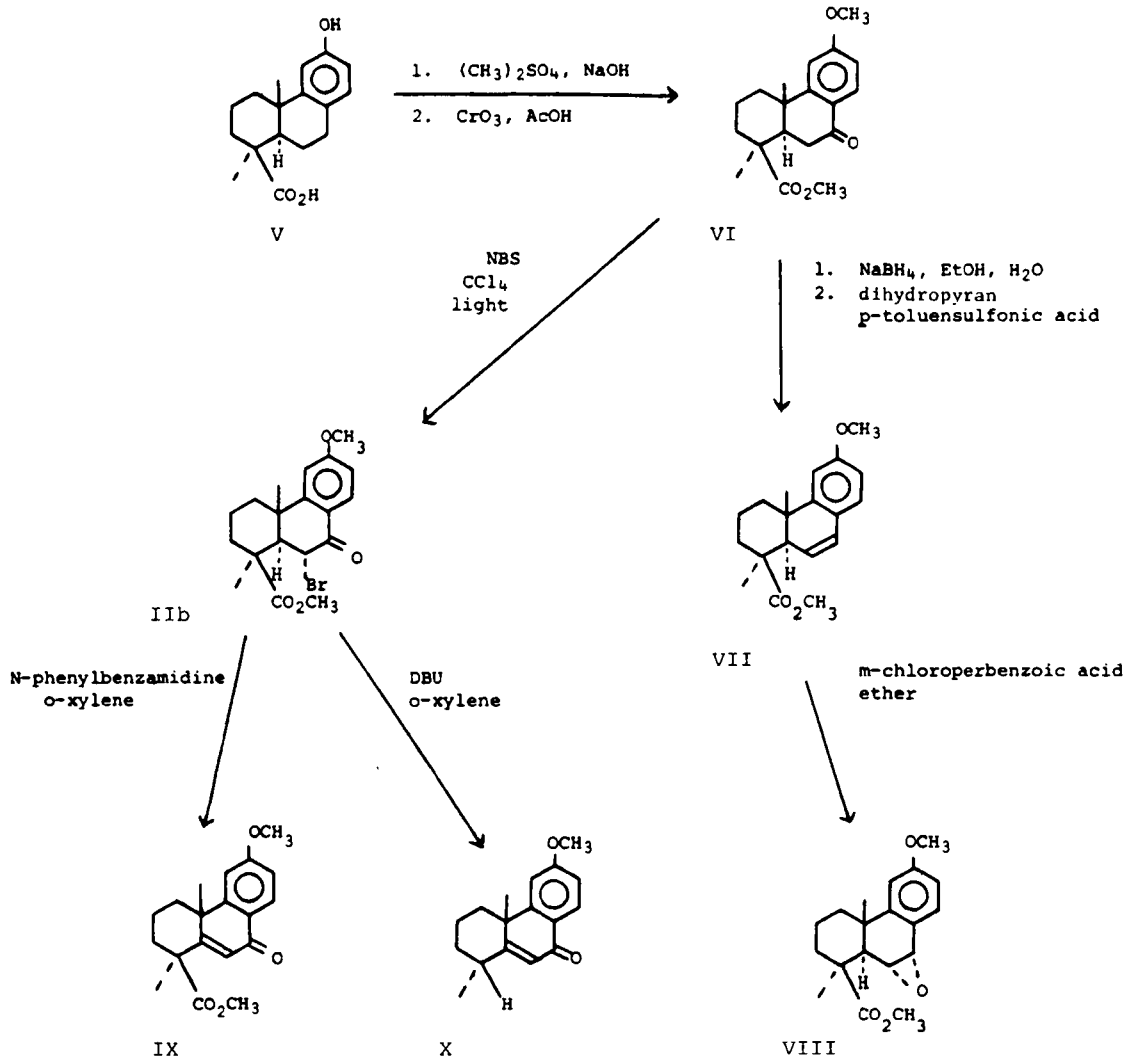

Scheme I

was removed by filtration. Evaporation of the solution *in vacuo* gave a pink syrup. The syrup was dissolved in ether (25 mL), followed by addition of 5% aqueous sodium chloride solution (10 mL). The aqueous layer was extracted several times with 50-mL portions of ether. The ether extracts were combined, washed twice with 5% aqueous sodium chloride (20 mL), dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. Crystallization from ether solution gave 8.8 g (88%) of the alcohol methyl *O*-methyl-7-hydroxypodocarpate as white crystals, mp 100–106°C. IR (KBr): 3500, 1715, 1610, and 1575 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.88 (s, 3, C—CH_3), 1.32 (s, 3, C—CH_3), 3.70 (s, 3, O—CH_3), 3.82 (s, 3, O—CH_3), 4.51 (m, 1, C*H*OH), and 6.83 ppm (m, 3, ArH). MS: *m/z* 318 (M^+); Calcd. for $C_{19}H_{26}O_4$: *m/z* 318 (M^+).

Anal.—Calc. for $C_{19}H_{26}O_4$: C, 71.76; H, 8.24. Found: C, 71.88; H, 8.35.

The aforementioned alcohol (8 g, 25.2 mmol), dihydropyran (5 mL), and 0.5 g of *p*-toluenesulfonic acid were heated at reflux for 8 h. The mixture was extracted with 200 mL of ether. The organic phase was washed with water, 5% aqueous sodium carbonate, and then water, and dried over anhydrous magnesium sulfate. Evaporation *in vacuo* yielded a yellow syrup, which was chromatographed (dry silica gel, 50:1). Elution with petroleum ether–methylene chloride gave excess dihydropyran in the early fractions and the unsaturated ester VII as a solid in the later fractions (monitored by GC). Compound VII was recrystallized from CCl_4–hexane (slow evaporation) to give light-brown crystals (6.4 g, 85%), mp 84–85°C. IR(KBr): 2950, 1720, and 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.86 (s, 3, C—CH_3), 1.35 (s, 3, C—CH_3), 3.75 (s, 3, O—CH_3), 3.88 (s, 3, O—CH_3), 6.51 (s, 2, vinylic), and 6.90 ppm (m, 3, ArH).

Anal.—Calc. for $C_{19}H_{24}O_3$: C. 75.96; H, 8.07. Found: C, 75.82; H, 8.14.

Epoxide VIII—To a solution of the unsaturated ester VII (5 g, 16.6 mmol) in 100 mL of dry ether was added slowly a solution of 3 g of *m*-chloroperbenzoic acid in 15 mL of dry ether with stirring. The solution was stirred for 6 h and then was allowed to stand overnight. After the addition of 10% $NaHCO_3$ (15 mL), the mixture was extracted with ether. The organic phase was washed (H_2O), dried ($MgSO_4$), and the solvent was removed to give a light-yellow syrup, which solidified on standing. Recrystallization from chloroform yielded 4.6 g (87%) of white crystals of VIII, mp 158–159°C. IR(KBr): 2950, 1725, and 1500 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.12 (s, 3, C—CH_3), 1.28 (s, 3, C—CH_3), 3.69 (s, 3, O—CH_3), 3.78 (s, 3, O—CH_3), and 6.88 ppm (m, 3, ArH).

Anal.—Calc. for $C_{19}H_{24}O_4$: C, 72.13; H, 7.65. Found: C, 72.16; H. 7.72.

Methyl *O*-Methyl-6-α-bromo-7-ketopodocarpate (IIb)—To ester VI (46 g, 0.2226 mol) was added 550 mL of carbon tetrachloride. After heating to dissolve the ketone, 28 g (0.157 mol) of *N*-bromosuccinimide was added, and the solution was allowed to stand (catalyzed by sunlight) for 96 h with occasional stirring. The solution was filtered and evaporated *in vacuo*. The residue (mp 123–124°C) was recrystallized five times from aqueous methanol to give

Table I—Activity of Podocarpic Acid Derivatives Against Human Epidermoid Carcinoma of the Nasopharynx *In Vitro* [a]

Compound	ED_{50} [b]	Slope [c]
IIb	3.1	−1.14
	3.4	−1.59
	4.0	−1.62
VII	7.9	−0.51
VIII	23.0	−0.95
IX	100.0	−0.21
X	21.0	−0.90

[a] Cell culture assay with a vehicle of dimethylformamide. [b] Expressed in micrograms per milliliter that inhibited 50% of the control dose. [c] The change of response from each one-log change of dose.

Table II—Activity of Podocarpic Acid Derivatives Against L1210 Lymphoid Leukemia

Compound	Host Mice[a]	Weight Difference,[b] g	Dose, mg/kg	T/C[c]
IIb	BDF_1	−0.8	400	101
		−0.2	200	101
		0.5	100	97
	CDF_1[d]	−0.8	200	112
		−0.2	100	100
		0.9	50	103
VII	BDF_1	0.7	400	87
		0.7	200	90
		−0.3	100	90
	CDF_1	−0.5	400	103
		−1.0	200	106
		−1.1	100	104
VIII	BDF_1	−0.2	400	101
		0.0	200	100
		−0.1	100	103
IX	BDF_1	1.1	400	87
		−0.3	200	89
		−0.3	100	92
X	BDF_1[e]	−0.4	400	90
		−0.4	200	88
		−0.4	100	97
		−0.2	50	100
		−0.1	25	100

[a] Intraperitoneal injections with saline with Tween 80 as a vehicle unless otherwise indicated. [b] Average weight change of test group minus average weight change of control animals. [c] Ratio of the mean survival time of the test animals to the control animals. [d] P388 lymphocytic leukemia screen. [e] Tested using another screen.

49 g (81%) of bromoketone IIb as a white crystalline solid, mp 142–144°C [lit. mp 142–144.5°C (1), 141–142°C (12), 135–137°C (13), and 123–126°C (14)]. IR (KBr): 2950, 1725, 1684, and 1604 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.85 (s, 3, C_{10}-CH_3) [lit. (7, 13) 0.85], 1.54 (s, 3, C_4-CH_3) [lit. (7, 13) 1.53], 3.70 (s, 3, OCH_3, ester), 3.84 (s, 3, OCH_3, ether), 5.80 (d, 1, J = 7 cps, CBrH) [lit. (7, 13) J = 7 cps], 6.85 (m, 2, ArH), and 7.80 ppm (d, 1, J = 9 cps, ArH).

Biological Procedures All biological tests were performed at the Cancer Chemotherapy National Service Center, Bethesda, Md. The general procedure, protocols, and data interpretation used at the National Cancer Institute have been published previously (15, 16).

REFERENCES

(1) R. H. Bible and M. Grove (to G. D. Searle and Co.) U.S. Pat. No. 2,753,357, July 3, 1956; *Chem. Abstr.*, **51**, 2869d (1957).

(2) M. N. Galbraith, D. H. S. Horn, J. M. Sasse, and D. Adamson, *Chem. Commun.*, **1970**, 170.

(3) J. M. Sasse, M. N. Galbraith, D. H. S. Horn, and D. A. Adamson, in "Plant Growth Substances, 1970," D. J. Carr, Ed., Springer Verlag, 1972, p. 430.

(4) M. N. Galbraith, D. H. S. Horn, and J. M. Sasse, *Chem. Commun.*, **1971**, 1362.

(5) R. F. Bryan and P. M. Smith, *J. Chem. Soc.* (Perkin II), **1975**, 148.

(6) G. R. Clark and T. N. Waters, *J. Chem. Soc.* (C) **1970**, 887.

(7) J. F. Cutfield, T. N. Waters, and G. R. Clark, *J. Chem. Soc.* (Perkin II), **1974**, 150.

(8) E. J. Parish and D. H. Miles, *J. Org. Chem.*, **38**, 3800 (1973).

(9) E. J. Parish and D. H. Miles, *J. Org. Chem.*, **38**, 1223 (1973).

(10) I. R. Sherwood and W. F. Short, *J. Chem. Soc.* **1938**, 1010.

(11) E. Wenkert, A. Fuchs, and J. D. McChesney, *J. Org. Chem.*, **30**, 2931 (1965).

(12) A. K. Bose, M. S. Manhas, and R. C. Cambie, *J. Org. Chem.*, **30**, 501 (1965).

(13) A. E. Lickei, A. C. Rieke, and D. M. S. Wheeler, *J. Org. Chem.*, **32**, 1647 (1967).

(14) E. Wenkert, *et al.*, *Can. J. Chem.*, **41**, 1924 (1963).

(15) R. I. Geren, N. H. Greenberg, M. M. MacDonald, A. M. Schumacker and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**(2), 1 (1972).

(16) "Screening Date Summary Interpretation and Outline of Current Screen," Drug Evaluation Branch Instruction Booklet 14, Division of Cancer Treatment, National Cancer Institute, Bethesda, 1978.

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Isocratic Liquid Chromatographic Method for the Determination of Amoxapine and its Metabolites

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Abstract □ An isocratic reverse-phase liquid chromatographic method for the determination of amoxapine and its major metabolites in human plasma utilizing UV detection is described. Plasma samples were extracted with ethyl acetate after pH adjustement. The reconstituted extracts were injected onto a cyanopropylsilane column and eluted with a mobile phase consisting of 65% acetonitrile and 35% sodium acetate buffer 0.03 M and pH 6. The minimum detectable limit was <10 ng/mL of plasma. Possible interferences from other drugs which might be administered concurrently were studied. The reproducibility and precision of the method are demonstrated by the analysis of samples containing 25–600 ng/mL of plasma. The method is being applied successfully in our laboratory for the analysis of plasma from patients receiving amoxapine.

Keyphrases □ Amoxapine—isocratic HPLC method, determination of metabolites □ Metabolites—isocratic HPLC method, determination of amoxapine

Amoxapine[1], 2-chloro-11-(1-piperazinyl)dibenz[*b,f*]-[1,4]oxazepine, a relatively new antidepressant of the dibenzoxazepine class, has been shown to be a potent antidepressant (1–3). The relationship between tricyclic antidepressant plasma concentrations and therapeutic response has been studied extensively, with inconclusive results (4, 5). The side effects of tricyclic antidepressants have also been correlated to plasma concentrations with mixed results (6). Since the major metabolic pathway of amoxapine is hydroxylation (7), monitoring therapeutic levels of the parent drug and its active

[1] Lederle Laboratories, Pearl River, N.Y.

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