of Ac_2O. After 2 days the solution was poured into ice–H_2O, and the solid was filtered, recrystallized from C_6H_6–cyclohexane, and chromatographed on 5 g of silica gel. Elution with EtOAc–$CHCl_3$ gave **8**, identified by nmr and chromatography.

3′-Thymidinyl 5′-Thymidinyl Carbonate (10).—An 80% HOAc (50 ml) solution of 2.0 g (2.7 mmoles) of **9** was refluxed for 10 min, poured into 200 ml of ice–H_2O, and filtered. Evaporation and recrystallization of the residue from MeOH gave 0.7 g (50%) of **10**, mp 205–210°, softens 137–150°; ascending chromatography in NH_4HCO_3 (16%) gave an R_f of 0.71. The nmr (DMSO-d_6) showed the expected resonance signals, similar to those found in **9**. *Anal.* ($C_{21}H_{26}N_4O_{11}$) C, H, N.

A sample (0.7 g, 1.37 mmoles) of **10** was heated in 50 ml of dry pyridine containing 0.7 g (2.75 mmoles) of trityl chloride for 2.5 hr. The solution was poured into 300 ml of ice–H_2O and filtered, and the solid was dissolved in $CHCl_3$, dried ($MgSO_4$), and chromatographed on 45 g of silica gel. Elution with 4% MeOH–$CHCl_3$ gave 0.22 g of a solid identical with **9** by melting point and ascending chromatography in *i*-PrOH–H_2O (4:6), R_f 0.78.

3′-(5′-Diphenylphosphorylthymidinyl) 5′-Thymidinyl Carbonate (11).—A cold (0°) solution of 100 mg (0.20 mmole) of **10** in 2 ml of dry pyridine was treated with 200 mg (0.75 mmole) of diphenyl phosphorochloridate[13] and maintained at 5° for 12 hr. The solvent was evaporated and the residue was dissolved in $CHCl_3$, washed with H_2O, dried, and chromatographed on 4 g of silica gel. Elution with CH_2Cl_2 containing increasing amounts of MeOH gave **12** and finally 0.027 g (25%) of **11** as glasses characterized by nmr.

It was found in subsequent reactions that the monophosphorylated product **11** could be separated from **12** by fractional crystallization from $CHCl_3$. *Anal.* (**11**, $C_{33}H_{35}N_4O_{14}P \cdot H_2O$) C, H, N, P.

Acetylation of 17 mg (0.023 mmole) of **11** was accomplished using 23 mg (0.23 mmole) of Ac_2O in 1.5 ml of dry pyridine. After 12 hr at 25° the solution was poured into ice–H_2O, extracted with $CHCl_3$, dried ($MgSO_4$), and evaporated to a glass. The nmr ($CDCl_3$) showed the acetyl methyl protons at 2.10 ppm and the two 3′-methynes at 5.30 ppm.

3′-(5′-Monophosphorylthymidinyl) 5′-Thymidinyl Carbonate (14).—An EtOH (5 ml) solution containing 100 mg (0.13 mmole) of **11** was added to prereduced PtO_2 (80 mg) in EtOH and reduced at atmospheric pressure for 18 hr; slightly more than the theoretical amount of H_2 was absorbed. Filtration and evaporation gave the product, mp 185–200°. *Anal.* ($C_{21}H_{27}N_4O_{14}P \cdot 2H_2O$) C, H; N: calcd, 8.95; found, 8.45.

3′-Thymidinyl 5′-(5-Fluoro-2′-deoxyuridinyl) Carbonate (15).—A C_6H_6 solution (75 ml) of the chloroformate **6** prepared from 1.5 g (3.1 mmoles) of 5′-O-tritylthymidine was added slowly (45 min) to a cold solution (0–5°) of 0.45 g (1.8 mmoles) of 5-fluoro-2′-deoxyuridine in 30 ml of dry pyridine. After stirring 12 hr, 50 ml of H_2O was added and the mixture was extracted with three 100-ml portions of $CHCl_3$. After drying and concentrating, the residue was chromatographed on 60 g of silica gel. Elution with $CHCl_3$ and 4% MeOH–$CHCl_3$ gave 0.56 g of the trityl product which was rechromatographed on 25 g of silica to remove minor impurities.

A solution of 0.447 g (0.59 mmole) of the tritylated compound was refluxed for 10 min in 20 ml of 80% HOAc, poured into 200 ml of ice–H_2O, and extracted with two 150-ml portions of C_6H_6. The residue of the aqueous solution was evaporated and washed with MeOH to give **15** as a solid; recrystallization from H_2O–MeOH gave 0.124 g (17%), mp 220–224°, uv, λ_{max}^{EtOH} 266 mμ. *Anal.* ($C_{20}H_{23}FN_4O_{11}$) C, H, F, N.

3′-(5′-Fluoro-2′-deoxyuridinyl) 5′-Thymidinyl Carbonate (18).—$COCl_2$ was passed for 45 min into a cold (0–10°) solution of 3.5 g (7.1 mmoles) of 5′-O-trityl-5-fluoro-2′-deoxyuridine[19] in 100 ml of dry THF containing 0.56 g (7 mmoles) of dry pyridine. After $COCl_2$ treatment, the mixture was filtered and concentrated (under vacuum) to 20 ml. This solution of **16** was added slowly to a cold (5°), stirred solution of 1.5 g (6.2 mmoles) of thymidine in 20 ml of dry pyridine. After 18 hr at room temperature the solution was poured into 300 ml of ice–H_2O. The aqueous solution was decanted from the heavy precipitate and the precipitate was dissolved in MeOH. Repeated dissolution and evaporation afforded 5.2 g of white semisolid which was chromatographed on 60 g of silica gel (Brinkman 0.20–0.05 mm). Elution with $CHCl_3$ and $CHCl_3$–2% MeOH removed starting materials and impurities. Elution with $CHCl_3$–4% MeOH afforded 3.21 g (68% based on thymidine) of 3′-(5′-O-trityl-5-fluoro-2′-deoxyuridinyl) 5′-thymidinyl carbonate (**17**). *Anal.* ($C_{39}H_{37}FN_4O_{11}$) C, H, F, N.

A solution of 0.95 g (1.2 mmoles) of the tritylated compound **17** was refluxed for 10 min in 20 ml of 80% HOAc, poured into 200 ml of ice–H_2O, and extracted with two 100-ml portions of C_6H_6. The aqueous solution was then evaporated *in vacuo*, washed with MeOH, and filtered to give 0.6 g (93%) of **18** as a white solid, mp 155–160°. *Anal.* ($C_{20}H_{23}FN_4O_{11}$) C, H, F, N.

(19) J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963).

Experimental Tumor Inhibitors. Antitumor Activity of Esters of ω-Aryl-ψ-nitro-ψ-alken-1-ol and Related Compounds[1]

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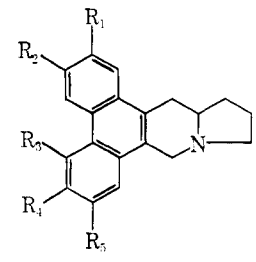
Preparation of a series of 5-substituted 4-nitro-4-penten-1-ol acetate and related analogs is described. Many compounds in this category demonstrated confirmed inhibitory activity against Walker carcinosarcoma 256 in preliminary biological testing. Structure–activity study indicated that the nitroalkene portion of the side chain is essential for the oncolytic property. The relative activity and toxicity of these compounds are dependent on the length of the aliphatic side chain and substitution at the terminal positions.

In connection with a structure–activity study of the alkaloids tylocrebrine (Ia) and tylophorine (Ib), which showed anticancer activity against leukemia L1210,[2] the phenanthro[9,10:6′,7′]indolizidine[3] nucleus (Ic) was prepared in this laboratory. Compound



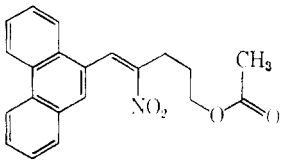
Ia, R_1, R_2, R_3, R_4 = OCH_3; R_5 = H
b, R_1, R_2, R_4, R_5 = OCH_3; R_3 = H
c, R_1, R_2, R_3, R_4, R_5 = H

(1) (a) The investigation is supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, of the National Institutes of Health, Public Health Service (Contract PH-43-65-94). (b) Presented in part before the Division of Medicinal Chemistry, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, N-59.

(2) E. Gellert and R. Rudzats, *J. Med. Chem.*, **7**, 361 (1964).

(3) T. R. Govindachari, M. V. Lakshmikantham, K. Nagarajan, and B. R. Pai, *Tetrahedron*, **4**, 311 (1958).

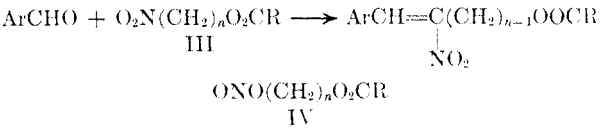
Ic does not retain the original activity exhibited by the methoxy derivatives. However, one of the intermediates leading to Ic, 4-nitro-5-(9-phenanthryl)-4-penten-1-ol acetate (II), demonstrated reproducible activity against the Walker carcinosarcoma 256 tumor system.



II

A number of nitro compounds of natural and synthetic origin were found to have biological activity.[4,5] A search of the literature revealed that, while some compounds containing a nitro function have long been recognized as carcinogens,[6] a limited number of compounds with nitro groups attached directly to aromatic ring systems possess tumor-inhibitory activity.[7-10] The antitumor property of nitroalkenes, to our knowledge, has not yet been described. Rather, these compounds were reported to have fungicidal,[11-13] antibacterial,[14-16] insecticidal,[15-17] and rodent-repelling[18] characteristics.

In order to better understand the nature of the antitumor activity of II, a structural modification study has been initiated. In general, compounds of this type can be prepared by the condensation of aromatic aldehydes with esters of ω-nitro-1-alkanol in acetic acid.



III IV

The nitro esters III were prepared by the treatment of the corresponding halogenated esters with silver nitrite.[19] The nitrite esters IV, formed as by-products, can be readily detected and separated[20] (see Figure 1) and subsequently isolated by fractional distillation.

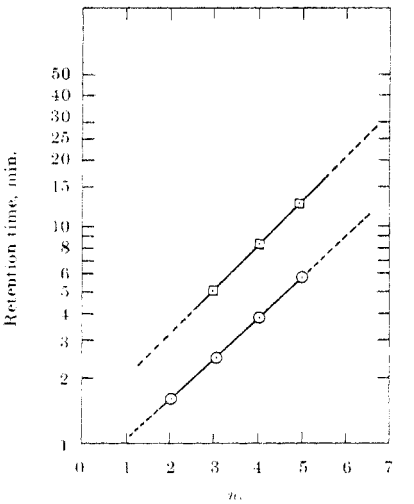
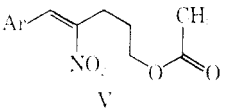


Figure 1.—Gas chromatography of ω-nitro and ω-nitrite esters. Column, Carbowax 20, 1 m; temperature, 175°; helium flow rate, 50 ml/min; instrument, Perkin-Elmer. ◈ = $O_2N(CH_2)_nOOCCH_3$, ⊙ = $ONO(CH_2)_nOOCCH_3$. The ir spectrum of the former compound showed absorption at 6.4 μ for the NO_2 group, and of the latter compound showed absorption at 6.1 μ for the ONO group.

1. Variation of the Aryl Portion.—The phenanthrene moiety was replaced by other aromatic systems. These



V

compounds were usually obtained in 10–40% yield by treatment of the appropriate aldehydes with 4-nitrobutyl acetate in the presence of NH_4OAc in AcOH. (Under similar reaction conditions, diphenylacetaldehyde, which possesses an α-hydrogen, reacted with NH_4OAc to form 2,2,2′,2′-tetraphenyldivinylamine.[21,22]) The 2-naphthyl analog was shown to be more active than the original compound (II) against Walker 256. In the case of various phenyl-substituted compounds, the antitumor activity becomes a function of the nature of substituents (see Table I).

2. Modification of Functional Groups on the Side Chain.—Reduction of the nitroalkene moiety by $LiAlH_4$ yielded a saturated amino alcohol VIa. This compound and its diformyl derivative VIb (both are intermediates for the synthesis of Ic) failed to retain the antitumor activity of the original compound.

(4) Z. Eckstein, *Oesterr. Chemiker-Ztg.*, **66**, 111 (1965); *Chem. Abstr.*, **63**, 18854*h* (1965).

(5) M. Movrin, *Farm. Glasnik*, **18**, 68 (1962); *Chem. Abstr.*, **58**, 3268*h* (1963)

(6) See, for example, (a) W. Nakahara, F. Fukuoka, and T. Sugimura, *Gann*, **48**, 124 (1957); (b) S. Laham, *Can. J. Biochem. Physiol.*, **38**, 1383 (1960); (c) J. A. Miller and E. C. Miller, *Cancer Res.*, **25**, 1292 (1965).

(7) H. Katae, H. Iwana, Y. Takase, and M. Shimizu, *Arzneimittel-Forsch.*, **17**, 1030 (1967).

(8) K. Miura, M. Ikeda, T. Oohashi, I. Okada, and Y. Igarashi, *J. Pharm. Soc. Japan*, **84**, 341, 537 (1964).

(9) (a) A. Ledochowski, *Zeszyty Nauk. Politech. Gdansk., Chem.*, 3 (1966); *Chem. Abstr.*, **68**, 76653*q* (1968); (b) Starogardzkie Zaklady Farmaceu. "polfa," French Patent 1,458,183 (Nov 10, 1966); *Chem. Abstr.*, **68**, 39493*s* (1968).

(10) P. B. Ghosh and M. W. Whitehouse, *J. Med. Chem.*, **11**, 305 (1968).

(11) E. W. Bousquet, J. E. Kirby, and N. E. Searle, U. S. Patent 2,335,384 (Nov 30, 1943); *Chem. Abstr.*, **38**, 2834 (1944).

(12) (a) P. W. Brian, J. F. Grove, and J. C. McGowan, *Nature*, **158**, 876 (1946); (b) J. C. McGowan, P. W. Brian, and H. G. Hemming, *Ann. Appl. Biol.*, **35**, 25 (1948).

(13) F. C. Bocobo, A. C. Curtis, W. D. Block, E. R. Harrell, E. E. Evans, and R. F. Haines, *Antibiol. Chemotherapy*, **6**, 385 (1956).

(14) O. Schales and H. A. Graefe, *J. Am. Chem. Soc.*, **74**, 4486 (1952).

(15) (a) M. Koremura, H. Oku, T. Shono, and T. Nakanishi, *Takamine Kenkyusho Nempo*, **13**, 198 (1961); (b) M. Koremura, *ibid.*, **13**, 205, 212, 228 (1961); (c) M. Koremura, H. Oku, H. Nakao, T. Shono, and Y. Morisawa, *ibid.*, **13**, 216 (1961); (d) M. Koremura and Z. Hattori, *ibid.*, **13**, 222 (1961).

(16) (a) M. Koremura, *Nippon Nogeikagaku Kaishi*, **36**, 473, 552, 557 (1962); (b) M. Koremura and K. Tomita, *ibid.*, **36**, 479 (1962).

(17) A. W. A. Brown, D. B. W. Robinson, H. Hurtig, and B. J. Wenner, *Can. J. Res.*, **26D**, 177 (1948).

(18) R. J. Harker, U. S. Patent 2,889,246 (June 2, 1959); *Chem. Abstr.*, **53**, 17414*i* (1959).

(19) Silver nitrite, rather than sodium nitrite, is preferred for the synthesis of nitro esters of the general structure $O_2N(CH_2)_xOOCR$. See N. Kornblum, *Org. Reactions*, **12**, 101 (1962).

(20) The presence of these esters can be detected by ir and gas chromatography. Nitro esters usually show a strong ir band at 6.5 μ. Gas chromatographic analysis revealed that the nitro esters always showed a longer retention time than the corresponding nitrite esters. When the log values of the retention time of the homologous series of these two esters were plotted against the number of carbon atoms of the straight chain, two straight and parallel lines resulted. This relationship can be used conveniently for detection and verification of new esters.

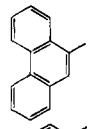
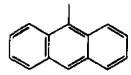
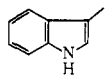
(21) W. Krabbe and K. H. Schmidt, *Ber.*, **72B**, 381 (1939).

(22) B. Witkop, *J. Am. Chem. Soc.*, **78**, 2873 (1956).

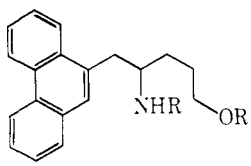
TABLE I

5-SUBSTITUTED 4-NITRO-4-PENTEN-1-OL ACETATE[a]

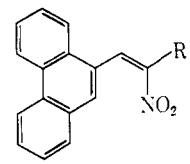
$$RCH{=}C(CH_2)_3OOCCH_3$$
$$\quad\quad|$$
$$\quad NO_2$$

No.	R	Mp, °C	Yield,[b] %	Formula[d]	Uv max[c] λ, mμ	Uv max[c] Log ε	Antitumor act. against WA 256 Dose, mg/kg	Survivors	Wt dif T − C	Tumor T/C
1		90–91	35	$C_{21}H_{19}NO_4$	340	3.87	800	5/6	−23	0.13
							400	20/24	−19	0.31
							200	6/6	−12	0.34
							100	6/6	−8	0.73
2		78–80	22	$C_{17}H_{17}NO_4$	324	4.09	400	2/6	...	...
							200	39/42	−12	0.26
							100	6/6	−4	0.71
3		101–103	20	$C_{21}H_{19}NO_4$	348	3.58	400	7/7	−2	0.85
4	C_6H_5	49–51	34	$C_{13}H_{13}NO_4$	305	4.02	50	6/6	−12	1.11
5	p-$CH_3CO_2(CH_2)_3C(NO_2){=}CHC_6H_4$	87–89	10	$C_{20}H_{24}N_2O_8$	330	4.31	400	0/6	...	...
							200	17/18	−20	0.25
							100	6/6	−16	0.24
							50	6/6	−5	0.70
6	p-$CH_3C_6H_4$	28–30	28	$C_{14}H_{17}NO_4$	318	3.94	400	6/6	−2	0.80
7	p-$CH_3OC_6H_4$	46–48	10	$C_{14}H_{17}NO_5$	340	4.19	400	6/6	5	1.01
8	3,4-$(CH_3O)_2C_6H_3$	62–64	10	$C_{15}H_{19}NO_6$	360	4.13	85	16/18	−8	0.44
9	3,4-$(CH_2O_2)C_6H_3$	90–92	21	$C_{14}H_{15}NO_6$	359	4.20	400	3/6	...	0.87
10	p-$(CH_3)_2NC_6H_4$	69–71	23	$C_{15}H_{20}N_2O_4$	416	4.36	400	12/12	−10	0.52
11	m-$NO_2C_6H_4$	72–74	21	$C_{13}H_{14}N_2O_6$			200	0/7	...	...
12	p-FC_6H_4	71–73	25	$C_{13}H_{14}FNO_4$	306	4.07	200	0/7	...	...
13	p-ClC_6H_4	69–71	31	$C_{13}H_{14}ClNO_4$	305	4.19	400	3/6	−2	...
							200	4/6	−3	0.68
14	3,4-$(Cl)_2C_6H_3$	72–74	26	$C_{13}H_{13}Cl_2NO_4$	301	3.93	50	5/6	−12	0.40
15	p-BrC_6H_4	68–69	32	$C_{13}H_{14}BrNO_4$	310	3.97	340	0/6	...	...
							170	1/7	−4	0.10
							85	11/12	−9	0.40
							43	6/6	−7	1.01
16		140–142	22	$C_{15}H_{16}N_2O_4$	396	4.23	100	4/6	−2	1.06

[a] All test results were provided by the Cancer Chemotherapy National Service Center of the National Cancer Institute. For an explanation and criteria of test data, see *Cancer Chemotherapy Rept.*, **25**, 1 (1962). [b] Purified compounds. [c] Characteristic conjugation absorption maxima (spectra taken in ethanol). [d] All compounds analyzed correctly for C, H, N.



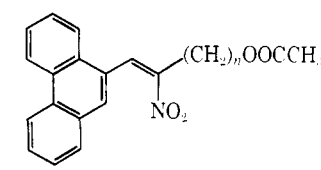
VIa, R = H
b, R = CHO



VIIa, R = H
b, R = $(CH_2)_2CH_3$
c, R = $(CH_2)_3OOCC_6H_5$

2-Nitro-1-(9-phenanthryl)ethane (VIIa), 2-nitro-1-(9-phenanthryl)-1-pentene (VIIb), and 4-nitro-5-(9-phenanthryl)-4-penten-1-ol benzoate (VIIc) were also prepared. Compound VIIa was obtained by the base condensation of nitromethane and 9-phenanthrenecarboxaldehyde in ethanol, whereas compounds VIIb and VIIc were obtained by the usual NH_4OAc–AcOH method. Compound VIIb possessed good activity against WA-256 at 400 mg/kg, but severe body weight loss was noted. Replacement of the acetate of II by a benzoate group, as in the case of VIIc, does not retain the original activity. Compound VIIa showed only marginal activity but a closely related compound, 1-(2-naphthyl)-2-nitroethene,[15c] demonstrated good inhibitory activity against WA carcinosarcoma 256 (see Table II).

3. Variation of the Length of the Side Chain.—The



VIII

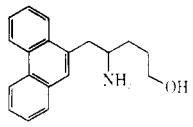
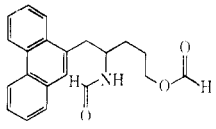
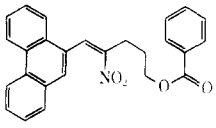
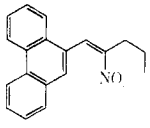
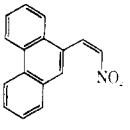
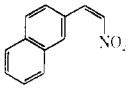
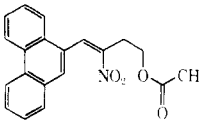
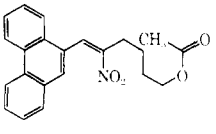
yield of ω-nitroacetates, which were prepared from silver nitrite and the corresponding ω-bromoacetates, decreases rapidly as the length of the chain is decreased. Practically no nitro ester was obtained when n is less than 4. In the case of 2-bromoethyl acetate (IX, n = 2), the nitrite ester (XI, n = 2) was the only product isolated.

$$\underset{\text{IX}}{Br(CH_2)_nOOCCH_3} \xrightarrow{AgNO_2} \underset{\text{X}}{O_2N(CH_2)_nOOCCH_3} + \underset{\text{XI}}{ONO(CH_2)_nOOCCH_3}$$

Preparations of X where n = 3 was achieved by the reaction of $AgNO_2$ with 3-bromopropanol followed by acylation (Ac_2O) of the resulting 3-nitropropanol.

TABLE II

STRUCTURAL ANALOGS OF 4-NITRO-5-(9-PHENANTHRYL)-4-PENTEN-1-OL ACETATE[a]

No.	Structure	Mp, °C	Yield,[b] %	Formula[c]	Uv max[c] λ, mμ	Uv max[c] Log ε	Antitumor act. against WA 256 Dose, mg/kg	Survivors	Wt dif T − C	Tumor T/C
17		118–120	26	$C_{19}H_{21}NO$			400	0/6	...	...
							100	6/6	−11	1.00
18		145–147	55	$C_{21}H_{21}NO_3$			200	6/6	−9	0.63
19		117–118	38	$C_{26}H_{21}NO_4$	342	3.89	400	7/7	−3	0.91
20		80–82	39	$C_{19}H_{17}NO_2$	338	3.72	400	24/25	−23	0.22
21		173–175	56	$C_{16}H_{11}NO_2$[d]	360	4.07	400	13/13	−13	0.46
22		121–123	60	$C_{12}H_9NO_2$[e]	325	3.90	400	2/6	−19	...
							200	21/24	−13	0.11
							100	10/12	−9	0.32
							50	6/6	−2	0.79
23		100–101	30	$C_{20}H_{17}NO_4$	340	3.86	400	3/6	−13	0.24
							200	0/6	...	...
							50	5/6	−2	0.73
24		69–71	10	$C_{22}H_{21}NO_4$	340	3.89	800	4/6	−22	0
							400	4/6	−20	0.11
							200	46/51	−11	0.38
							100	18/18	−4	0.68
							50	12/12	−3	0.78

[a–c] See corresponding footnotes in Table I. [d] See ref 24. [e] See ref 15c. [f] See footnote *d* in Table I.

Compound VIII where $n = 4$ possessed confirmed activity against WA-256 comparable to the original compound ($n = 3$). When $n = 2$, the resulting compound VIII became toxic (see Table II).

The foregoing preliminary screening results can be summarized as follows. (1) Among derivatives of 5-substituted 4-nitro-4-penten-1-ol acetate, β-naphthyl and 9-phenanthryl derivatives possess confirmed antitumor activity against Walker carcinosarcoma 256. Derivatives with unsubstituted phenyl (**4**) or phenyl substituted with electron-donating groups (**7–10**) do not retain the original activity, whereas phenyl substituted with electron-withdrawing groups seemed to possess some activity. 5,5′-*p*-Phenylenebis(4-nitro-4-penten-1-ol) diacetate (**5**), which can be considered as a compound in the last category, possesses confirmed activity against WA 256. (2) The nitroalkene portion of the side chain is essential for the antitumor activity. This is demonstrated by the facts that the reduced compounds (**17** and **18**) are inactive, whereas **20–22** are active. (3) In considering the length of the aliphatic side chain, increased activity was observed with a straight chain of six carbons (**24**), whereas increased toxicity and decreased activity was shown with a straight chain of four carbons (**23**). (4) Substitution of an acetate by a benzoate group (**19**) depressed the original activity. (5) β-Naphthyl derivatives seemed to have better activity than the corresponding 9-phenanthryl derivatives (compare **1** *vs.* **2**, and **21** *vs.* **22**).

Experimental Section[23]

3-Nitropropyl Acetate (X, $n = 3$).—To a stirred suspension of 416 g (2.7 moles) of $AgNO_2$ in 1 l. of EtOAc was added dropwise, at 0°, 250 g (1.8 moles) of 3-bromo-1-propanol. After the addition was complete (*ca.* 2 hr), the reaction mixture was stirred at room temperature for 2 days. It was then heated gently on a steam bath for 1 hr, cooled, and the Ag salt was removed by filtration. The filtrate was concentrated under reduced pressure to give 179 g of the crude 3-nitro-1-propanol (67% yield by gc analysis: 125 g of 3-nitro-1-propanol). To the crude intermediate was added 500 ml of Ac_2O. The resulting mixture was heated on a steam bath for 4 hr and evaporated under reduced

(23) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The uv absorption spectra were determined with a Beckman DK-2 spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

pressure to give 232 g of an oily product. It was fractionally distilled *in vacuo* and the fraction boiling at 85–95° (2.5 mm) was collected. Gc analysis indicated an over-all yield of 33% (87 g) of product. An analytical sample had bp 93° (2.5 mm), ir 6.5 μ (NO_2). *Anal.* ($C_5H_9NO_4$) C, H.

4-Nitrobutyl Acetate (X, n = 4).—To a stirred suspension of 270 g (1.75 moles) of $AgNO_2$ in 400 ml of anhydrous Et_2O was added dropwise, at 0°, 235 g (1.20 moles) of 4-bromobutyl acetate. After the addition was complete (*ca.* 3 hr), the reaction mixture, protected from moisture and light, was stirred at 0° for 24 hr and then at room temperature for an additional 48 hr. The resulting mixture was filtered and the solid (AgBr) was washed with two 80-ml portions of Et_2O. The combined filtrate and washings were evaporated under reduced pressure to yield 196 g of a yellow oil. It was fractionally distilled to give 84.9 g (44% yield) of product and 75.1 g (38% yield) of the nitrite ester. (The yield and purity were determined by ir and gas chromatography.) Repeated fractionation of the nitro ester yielded an analytically pure compound, bp 103–105° (2.2 mm). *Anal.* ($C_6H_{11}NO_4$) C, H, N.

5-Nitroamyl acetate (X, n = 5), bp 124–126° (2.5 mm), was prepared in a similar fashion, yield 42%.

2-Bromoethyl Acetate and Silver Nitrite.—Under reaction conditions similar to those above, only the nitrite ester (9.7 g, 72% yield) was obtained from 16.7 g of 2-bromoethyl acetate and 23.2 g of $AgNO_2$. The product showed an ir band at 6.1 μ (ONO), and did not condense with an aromatic aldehyde. *Anal.* ($C_4H_7NO_4$) C, H, N.

General Procedure for the Condensation of Aromatic Aldehyde and ω-Nitro-ψ-alkan-1-ol Acetate.—A mixture of 0.1 mole of the appropriate aromatic aldehyde, 0.1 mole of 4-nitrobutyl acetate, and 0.12 mole of NH_4OAc in 140 ml of glacial AcOH was refluxed for 2 hr. The solvent was removed under reduced pressure and the residue was poured into 500 ml of ice–H_2O. The mixture was then extracted with four 200-ml portions of Et_2O. The combined ethereal extracts were washed with three 80-ml portions of H_2O and dried (Na_2SO_4). The solvent was then removed and the oily residue was triturated with a small amount of CH_3OH. On standing (or freezing in a Dry Ice–acetone bath) the product slowly separated as a yellow solid. It was filtered off and recrystallized from MeOH (see Table I).

2,2,2′,2′-Tetraphenyldivinylamine.—A mixture of 90.6 g (0.1 mole) of diphenylacetaldehyde, 24.2 g (0.15 mole) of 4-nitrobutyl acetate, 5.0 g (0.065 mole) of NH_4OAc in 300 ml of AcOH was refluxed for 2 hr. The reaction mixture was cooled. The resulting white solid was isolated by filtration, washed with two 10-ml portions of AcOH followed by two 10-ml portions of petroleum ether (bp 35–60°) to give 8.5 g (46% yield) of product, mp 145–147°, $\lambda_{max}^{C_2H_5OH}$ 358 μ (log ϵ 4.40). No ir C=O absorption band was noted. This product was identical with that prepared by dehydration of 2-hydroxy-2,2-diphenylethylamine[21] or by chemical reduction of 1,1-diphenyl-2-nitroethene.[22] The same product was obtained in quantitative yield by refluxing a mixture of 19.6 g of diphenylacetaldehyde and 11.6 g of NH_4OAc in 150 ml of AcOH.

1-Nitro-2-(9-phenanthryl)ethene.—The following procedure is a modification of that reported by Mosettig and May.[24] To a warm (30–40°) solution of 10.3 g (0.050 mole) of 9-phenanthrenecarboxaldehyde and 3.1 g (0.05 mole) of $MeNO_2$ in 200 ml of EtOH was added in 5 min, with stirring, 50 ml of 8% aqueous KOH. The solution was stirred for another 30 min and subsequently poured into 160 ml of 15% HCl with cooling and vigorous stirring. The resulting yellow solid was filtered off and washed (H_2O) to give 11.7 g of crude product, mp 140°. It was recrystallized (EtOH) to give 7.0 g of pure product, mp 173–175° (lit.[24] mp 173–173.5°).

Reduction of 4-Nitro-5-(9-phenanthryl)-4-penten-1-ol Acetate (II).—To 200 ml of THF containing 16 g of $LiAlH_4$ was added dropwise a solution of 15.6 g (0.045 mole) of II in 120 ml of THF. After the addition was complete, the mixture was stirred at room temperature for 16 hr and then decomposed (H_2O). The solid was filtered and washed with two 50-ml portions of THF. The combined filtrate and washings were evaporated (temperature <40°) to dryness *in vacuo*. To the residue was added, with stirring, 200 ml of Et_2O and 200 ml of C_6H_6. After being refrigerated overnight the white solid (6.8 g, mp 75–140°), which consisted of a mixture of oxazine and saturated amino alcohol, was collected by filtration. Fractional recrystallization of the white solid from 500 ml of C_6H_6 gave 1.0 g (8% yield) of 3-(9-phenanthrylmethyl)-3,4,5,6-tetrahydro-1,2-oxazine, mp 187–189° (lit.[3] mp 185°). The mother liquor, after being concentrated to 50 ml and diluted with 150 ml of Et_2O, deposited 3.2 g of 4-amino-5-(9-phenanthryl)pentanol, mp 118–120° (lit.[3] mp 120°).

4-Formamido-5-(9-phenanthryl)penten-1-ol Formate.—A solution of 6.5 g (0.018 mole) of the amino alcohol, obtained from the aforementioned $LiAlH_4$ reduction, in 100 ml of HCOOAc was stirred for 16 hr at room temperature. Excess anhydride was removed *in vacuo* to an oily residue. The residue was triturated with 200 ml of anhydrous Et_2O for 10 min and the resulting white solid (3.3 g) was collected by filtration, mp 145–148°. Recrystallization of 0.5 g of the solid from 50 ml of C_6H_6 gave 0.4 g of pure product, mp 147–149° (lit.[3] mp 145°).

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Potential Anticancer Agents. V. New Aromatic Nitrogen Mustards Related to 3-[N,N-Bis(2-chloroethyl)amino]-4-methylbenzoic Acid

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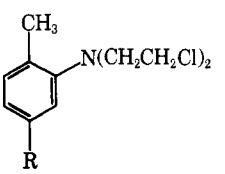
The synthesis of new esters and amides of 3-[N,N-bis(2-chloroethyl)amino]-4-methylbenzoic acid is described. New nitrogen mustards, derivatives of phosphoric and sulfonic aromatic acids, are also reported. A quantitative relation between hydrolysis rate of these N-mustards and basicity of precursor amines was established. Enhancement of the hydrolysis rate due to the steric effect of *o*-methyl groups was pointed out. Antitumor activity was tested against Jensen sarcoma, Walker 256 carcinosarcoma, and Guérin T8 carcinoma.

Previous studies on the relationship between chemical reactivity and antitumor properties in aromatic nitrogen mustards series led us to assign some special "carrier" properties to the benzoic acid structure.[1] Promising pharmacological and clinical results[2] obtained with 3-[N,N-bis(2-chloroethyl)amino]-4-methylbenzoic acid (Ia, R = CO_2H) prompted a closer examination of compounds I, in which the chemical re-



I

activity of the nitrogen mustard moiety is enhanced by an *o*-methyl substituent.[3]

(1) O. Costăchel, I. Niculescu-Duvăz, A. Cambanis, and G. Ciustea, Proceedings of the National Conference of Oncology, Bucharest, Nov 4–6, 1965.

(2) O. Costăchel and I. Mogos, *Oncol. Radiol.*, **7**, 255 (1968).

(3) I. Niculescu-Duvăz, M. Ionescu, A. Cambanis, M. Vitan, and V. Feyns, *J. Med. Chem.*, **11**, 500 (1968).