to I. Interest in this particular heterocyclic ring system and already been displayed in another laboratory.[6] However, the chlorooxazaphosphorine 2-oxide IV has not been previously made. In addition to the above-mentioned interests, IV was synthesized so that a comparison might be made between it and types II and III with respect to animal toxicity, antineoplastic activity, as well as reactivity toward amines.

The rate of the reaction of all of the above compounds was qualitatively determined by observing the rate of formation of triethylamine hydrochloride during the reactions with piperidine in nonpolar solvents containing triethylamine. It was found that III and IV were comparable in rate and faster than compounds of type II.

The structure proof of compounds of types II and III and of IV was based on their nitrogen analysis, characteristic infrared data (Table I), and the nitrogen analysis of their piperidine derivatives. The structure of compounds of type II was further characterized by comparing the product formed when paths A and B in Scheme IV were investigated. The identical product VIII was obtained, as shown by mixture melting points and identical infrared curves.

Biological.—According to all the available data, which is presented in Table II, none of the compounds showed any appreciable *in vivo* activity according to the criteria established by the Cancer Chemotherapy National Service Center.[7] The systems used were Friend virus leukemia, Hepatoma 129, Sarcoma 180, L1210 lymphoid leukemia, Adenocarcinoma 775, and Lewis lung carcinoma. On the other hand, IIb and IIc showed appreciable activity in cell culture (KB) of human epidermoid carcinoma of the nasopharynx.

Experimental Section

2-Chloro-1,3-bis(aralkyl)-1,3,2-diazaphosphorine 2-Oxides (II).—The synthesis of these compounds is typified by the preparation of 2-chloro-1,3-bis(*p*-methoxybenzyl)-1,3,2-diazaphosphorine 2-oxide. An ether solution of 10 g (0.032 mole) of the desired diamine and 6.5 g (0.064 mole) of triethylamine was added dropwise to 4.86 g (0.032 mole) of $POCl_3$ in 200 ml of cold ether as the mixture was stirred. The triethylamine hydrochloride formed at once and, after stirring the mixture for 1 hr, the hydrochloride was removed by filtration. The ether was removed under reduced pressure and 11.9 g (95% crude) of white solid was obtained. After one recrystallization from acetonitrile, 7.0 g of white solid melting at 151–152° was collected; over-all yield 55.7%.

2-Chloro-5-alkyl-5-nitro-1,3,2-dioxaphosphorinane 2-oxide (III) was synthesized by a procedure related to that used by Lanham.[8] The synthesis is typified by the production of 2-chloro-5-methyl-5-nitro-1,3,2-dioxaphosphorinane 2-oxide (IIIa). 2-Methyl-2-nitro-1,3-propanediol (20 g, 0.148 mole) was placed in 33 g (0.200 mole) of $POCl_3$. The solid diol went into solution after heating at 70° overnight. The solution was allowed to cool and a solid formed at once. This solid was collected on a sintered-glass funnel and washed with 200 ml of CCl_4 followed by 200 ml of petroleum ether (bp 30–60°). After drying overnight under vacuum the product weighed 31 g (97% yield). Recrystallization was carried out in acetonitrile to give 26 g of pure sample melting at 162–163°, over-all yield 82.0%.

2-Chlorohexahydro-1H,3H-pyrido[1,2-*c*][1,3,2]oxazaphosphorine 2-Oxide (IV).—Phosphorus oxychloride (5.9 g, 0.039 mole) was dissolved in 100 ml of anhydrous diethyl ether. A mixture of 5.0 g (0.039 mole) of 2-(2-hydroxyethyl)piperidine and 7.9 g (0.078 mole) of triethylamine in 50 ml of diethyl ether was added dropwise to the stirring solution, which was kept at 5°. A precipitate of triethylamine hydrochloride formed at once and after all the amine solution was added, the mixture was stirred at room temperature for 2 hr. At the end of the this time, 5 g of amine hydrochloride was removed by filtration. The ether was removed under reduced pressure to yield 8.0 g of crude product melting at 62–65°. One recrystallization from a 5:1 mixture of ethyl acetate–petroleum ether gave 5 g (62.5%) of pure product melting at 63–65°.

(6) L. Molnar and T. Wagner-Jauregg, Swiss Patent 387,639 (1965).

(7) *Cancer Chemotherapy Rept.*, **25**, 1 (1962). A compound is active against Walker 256 if it has a therapeutic index TI ≥ 4, where TI = LD_{10}/ED_{90}. A compound is confirmed active in (a) KB cell culture if the average $ED_{50} \geq 4$ μg ml for results from two laboratories; (b) Sarcoma 180, Lewis lung carcinoma, and solid Friend virus leukemia if the average T/C ≥ 42% in three confirming tests; and (c) lymphoid leukemia L1210 if T/C ≥ 125% in a confirmation test.

(8) W. M. Lanham, U. S. Patent 2,892,862 (1959).

Synthesis of Potential Antineoplastic Agents. XVI. Cyano Derivatives of 1,2,3,4-Tetrahydroquinoxaline and Related Compounds[1a,b]

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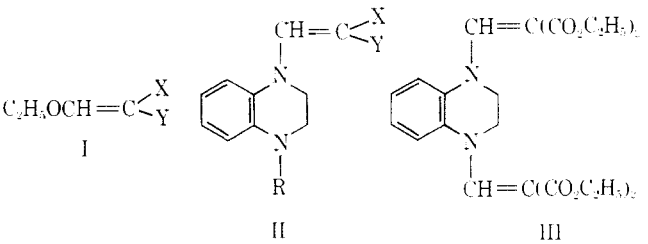
Cyano derivatives of both antimetabolites and alkylating agents have shown some promise as anticancer agents in preliminary studies.[2] This fact coupled with the observation[1a] that some derivatives of 1,2,3,4-tetrahydroquinoxaline showed activity in the KB line tissue culture screen prompted us to attempt to prepare derivatives of 1,2,3,4-tetrahydroquinoxaline containing a cyano group.

A convenient route to such compounds would appear to be the condensation[3,4] of compounds such as ethoxymethylenemalononitrile (I, X = Y = CN) with the tetrahydroquinoxaline. A series of model reactions were first run with I and 1,2,3,4-tetrahydroquinoline and 1,2,3,4-tetrahydroisoquinoline. The results are shown in Table I.



When these reactions were extended to 1,2,3,4-tetrahydroquinoxaline, it was found that the products obtained from the reactions with ethyl ethoxymethylenecyanoacetate (I, X = CN; Y = $CO_2C_2N_5$) and ethoxymethylenemalononitrile (I, X = Y = CN) were the monosubstituted derivatives II (R = H) (Table

(1) (a) Part XV: P. Schuyler, F. D. Popp, A. C. Noble, D. W. Alwani, and B. R. Masters, *J. Med. Chem.*, **9**, 704 (1966). (b) Supported in part by research grants from the American Cancer Society (T-177D) and from the National Cancer Institute, U. S. Public Health Service (CA 06606-03). Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965. (c) This work has been abstracted from the M. S. Thesis of P. S.

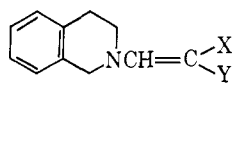
(2) For leading references see W. J. Burke, J. E. Brown, C. Weatherbee, and D. H. Curtis, *J. Med. Chem.*, **7**, 670 (1964).

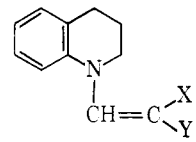
(3) E. A. Steck, *J. Org. Chem.*, **27**, 306 (1962).

(4) A. A. Santilli, W. F. Broce, and T. S. Osdene, *J. Med. Chem.*, **7**, 68 (1964).

TABLE I

DERIVATIVES OF TETRAHYDROQUINOLINE AND TETRAHYDROISOQUINOLINE



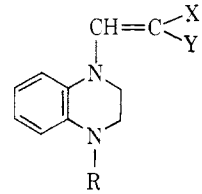


Tetrahydro base[a]	X	Y	Bp (mm) or mp, °C	Yield, %	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
Q	CN	CN	159–160	66	74.62	5.30	20.08	74.81	5.32	20.16
Q	$CO_2C_2H_5$	$CO_2C_2H_5$	210 (1.5)	51	67.31	6.98	4.62	67.13	7.02	4.74
Q	CN	$CO_2C_2H_5$	114–115	91	70.29	6.29	10.93	69.93	6.55	10.94
isoQ	CN	CN	122–123	76	74.62	5.30	20.08	74.73	5.22	20.18
isoQ	$CO_2C_2H_5$	$CO_2C_2H_5$	210 (1.1)	50	67.31	6.98	4.62	67.26	6.92	4.64
isoQ	CN	$CO_2C_2H_5$	108–110	95	70.29	6.29	10.93	70.39	6.31	10.86

[a] Q = 1,2,3,4-tetrahydroquinoline, isoQ = 1,2,3,4-tetrahydroisoquinoline.

TABLE II

DERIVATIVES OF TETRAHYDROQUINOXALINE

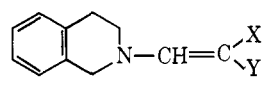


R	X	Y	Mp, °C	Yield, %	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
H	CN	CN	178–179[a]	49	68.56	4.80	26.66	68.35	4.72	26.94
H	CN	$CO_2C_2H_5$	121–122[a]	75	65.32	5.88	16.35	64.80	5.73	16.54
H	$CO_2C_2H_5$	$CO_2C_2H_5$	Oil[b]	18	63.15	6.62	9.21	63.32	6.75	9.11
$CH{=}C(CO_2C_2H_5)_2$	$CO_2C_2H_5$	$CO_2C_2H_5$	116–117[c]	50	60.75	6.37	5.90	60.63	6.51	5.79
$COCH_2CH_2Cl$	CN	CN	140–142[d]	75	59.90	4.36	18.63	59.79	4.39	18.65
$COCH_2CH_2Cl$	CN	$CO_2C_2H_5$	129–130[d]	82	58.87	5.23	12.12	58.88	5.08	12.00

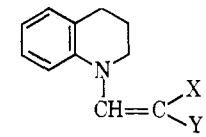
[a] Recrystallized from methanol. [b] Purified by chromatography. [c] Recrystallized from ethanol after separation from R = H, X = Y = $CO_2C_2H_5$ by chromatography. [d] Recrystallized from ethanol.

TABLE III

ANTINEOPLASTIC ACTION[a] OF



and



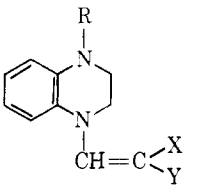
Base[b]	X	Y	KB cell culture[c] ED_{50}, μg/ml	KB cell culture[c] Slope	% (T/C)/dose, mg/kg LE[d]	8P[e]	DA[f]	Other
isoQ	CN	CN	3.4×10^1	−0.8	91/200	58/400	95/200	
isoQ	CN	$CO_2C_2H_5$	7.9×10^1	−0.4	90/200	71/200	91/100	38/10[g]
isoQ	$CO_2C_2H_5$	$CO_2C_2H_5$	3.9×10^1	−0.8	102/100	60/100	109/50	
Q	CN	CN	$M1.0 \times 10^2$		90/400	79/400	120/200	142/20[g]
Q	CN	$CO_2C_2H_5$	2.6×10^1	−1.1	97/200			127/200,[h] 76/250[i]
Q	$CO_2C_2H_5$	$CO_2C_2H_5$			93/200			75/200,[j] 93/200[k]

[a] Data from Cancer Chemotherapy National Service Center. [b] isoQ = tetrahydroisoquinoline, Q = tetrahydroquinoline. [c] ED_{50} = dose that inhibits 50% of control growth. Slope = difference in result for a tenfold difference in dose. [d] L1210 lymphoid leukemia. [e] P1798 lymphosarcoma. [f] Dunning leukemia (ascites). [g] HS1 human sarcoma (rat, egg). [h] Friend virus leukemia (solid). [i] Cystadenocarcinoma of the liver. [j] Lewis lung carcinoma. [k] Sarcoma 180.

II). That these compounds were II and not some structure bridged between the nitrogens was demonstrated by the appearance of an N–H stretching vibration in the infrared and by the fact that they reacted with 3-chloropropionyl chloride to give II (R = $COCH_2CH_2Cl$). In the reaction of diethyl ethoxymethylenemalonate (I, X = Y = $CO_2C_2H_5$) and 1,2,3,4-tetrahydroquinoxaline a product could not be isolated by the ordinary means of purification used in this series. When the reaction was run under more severe conditions and the resulting syrup chromatographed, II (R = H; X = Y = $CO_2C_2H_5$) and III were obtained.

The antineoplastic screening results for the compounds prepared are included in Tables III and IV. It can be seen that no outstanding activity is possessed by any of these compounds. It is of interest to note, however, that the two 3-chloropropionyl groups are apparently not necessary for the activity of

TABLE IV
ANTINEOPLASTIC ACTION[a] OF



X	Y	R	KB cell culture[b] ED_{50}, μg/ml	Slope	Sarcoma 180[c] SA[e]	Lewis lung carcinoma[d] LL[d]	L1210 lymphoid leukemia[e] LE[e]
					% (T/C)/dose, mg/kg		
CN	CN	H	$M1.0 \times 10^{-5}$		70/500	89/400	101/400
CN	$CO_2C_2H_5$	H	$M1.0 \times 10^{-5}$		89/500	79/400	90/400
CN	CN	$COCH_2CH_2Cl$	2.9×10^{-6}	−1.2	134/500	36/400	101/400
CN	$CO_2C_2H_5$	$COCH_2CH_2Cl$	5.5×10^{-6}	−0.5	64/500	71/350	93/350

[a] See footnote *a*, Table III. [b] See footnote *c*, Table III. [c] Sarcoma 180. [d] Lewis lung carcinoma. [e] L1210 lymphoid leukemia.

1,4-bis(3-chloropropionyl)-1,2,3,4-tetrahydroquinoxaline[1a] against KB cell culture since II (R = $COCH_2CH_2Cl$) is also active against this system.

Experimental Section[5]

β,β-Disubstituted N-Vinyltetrahydroquinolines and -isoquinolines.—A solution of 0.05 mole of 1,2,3,4-tetrahydroquinoline or 1,2,3,4-tetrahydroisoquinoline and 0.05 mole of the ethoxymethylene compound (I)[6] in 125 ml of methanol was refluxed for 1 hr and concentrated *in vacuo*. Solids were recrystallized from methanol and oils were distilled to give the compounds listed in Table I.

1-(β,β-Disubstituted vinyl)-1,2,3,4-tetrahydroquinoxaline and 1,4-Bis(β,β-dicarbethoxyvinyl)-1,2,3,4-tetrahydroquinoxaline.—As described above 0.05 mole of 1,2,3,4-tetrahydroquinoxaline and 0.1 mole of the ethoxymethylene compound (I) gave the compounds of the type II as indicated in Table II. In the case of diethyl ethoxymethylenemalonate two products were obtained and separated on acid-washed alumina.

1-(β,β-Disubstituted vinyl)-4-(3-chloropropionyl)-1,2,3,4-tetrahydroquinoxaline.—To a solution of II (R = H; X = CN; Y = CN or $CO_2C_2H_5$) in 125 ml of anhydrous chloroform at 0° was added dropwise, with constant stirring, a solution of 0.05 mole of 3-chloropropionyl chloride in 30 ml of anhydrous $CHCl_3$. The mixture was refluxed for several hours, filtered, and concentrated *in vacuo* to give compounds which are included in Table II.

(5) Analyses by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were taken in capillaries and are corrected.

(6) Initial samples of these compounds were generously donated by the Kay-Fries Chemicals, Inc.

Carcinostatic Sulfonic Acid Esters of Butyne- and Butane-1,4-diols[1a,b]

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The synthesis and evaluation of the anticancer properties of substituted benzenesulfonate esters of 1,4-butanediol and 1,4-butynediol was undertaken since esters of alkanesulfonic acids[2] have been found to be important anticancer compounds and, like nitrogen and sulfur mustards, are alkylating agents.[3] One sulfonic acid ester in particular has been shown to be an important therapeutic agent. This compound, 1,4-butanediol dimethanesulfonate (Ia) (known as Myleran® and Busulfan B®) was shown by Timmis and Haddow[4] to be effective in the management of granulocytic leukemia. It was also found to retard the growth of Adenocarcinoma 755, Glioma 26, and Brown-Pierce carcinoma[5] and was most active in Koller's[6] series on the effects of aliphatic sulfonate esters on the Walker carcinoma.

Although many studies have been made to elucidate the mechanism of action of alkylating agents, the literature is still filled with controversy. The following is a summary of some, although not all, of the studies which have been previously reported. Compound Ia in the *in vitro* studies of Parham and Wilbur[7] as in *in vivo* systems of Roberts and Warwick[8] appears to exhibit the same mechanism of action. The former workers followed the reaction of 1,4-butanediol dimethanesulfonate with the ethyl ester of cysteine in the presence of sodium hydroxide in ethanol and obtained the bisalkylated ethyl ester of cysteine and some tetrahydrothiophene. Parham and Wilbur[7] suggested a cyclic mechanism for the formation of tetrahydrothiophene from Ia and cysteine, and thought that this sulfur-stripping reaction might be responsible for the physiological activity of bifunctional alkylating agents in cancer chemotherapy. More recent work[9] with Ia and a number of mercaptans gave results quite analogous to those obtained from the reaction of Ia with the ethyl ester of cysteine. Meanwhile, Roberts and Warwick,[8] in an independent study of the reaction of Ia and cysteine *in vivo*, observed this same sulfur-stripping reaction. Subsequent studies,[10] in which carbon atoms of the alcohol portion of Ia were labeled with ^{14}C, have shown that it is metabolized in the mouse and excreted as 3-hydroxythiophene 1,1-dioxide.

(1) (a) This work was supported by Research Grant CA-06140 from the National Cancer Institute, Public Health Service. (b) Reported in part before the Medicinal Chemistry Division, 149th National Meeting, American Chemical Society, Detroit, Mich., March 1965. (c) Taken from the thesis of R. A. Earl which was submitted as partial fulfillment of the requirements for the Master of Arts Degree. (d) To whom inquiries should be sent.

(2) T. H. Goodridge, M. T. Flather, R. E. Harmon, and R. P. Bratzel, *Cancer Chemotherapy Rept.*, **No. 9**, 78 (1960).

(3) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. (Publishers) Ltd., London, 1962, p 111.

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(5) A. Gellhorn, A. Kells, and M. Golino, *Cancer Res. Suppl.*, **3**, 38 (1955).

(6) P. C. Koller, *Ann. N. Y. Acad. Sci.*, **68**, 789 (1958).

(7) W. E. Parham and J. M. Wilbur, Jr., *J. Am. Chem. Soc.*, **81**, 6071 (1959).

(8) J. J. Roberts and G. P. Warwick, *Nature*, **183**, 1509 (1959).

(9) W. E. Parham and J. M. Wilbur, Jr., *J. Org. Chem.*, **26**, 1569 (1961).

(10) J. J. Roberts and G. P. Warwick, *Nature*, **184**, 1288 (1959).