Acknowledgment.—The authors are indebted to Messrs. Jan Gyllander and Lembit Mikiver for skillful assistance in the synthetic work. Microanalyses were by Dr. Alfred Bernhardt, Microanalytical Laboratory, Mühlheim, Germany.

Dihydro-1,3-oxazines as Antitumor Agents

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Dihydro-*m*-benzoxazines were prepared by condensation of phenols, β-naphthol and 7-hydroxycoumarins with formaldehyde and primary amines. The products were quaternized or cleaved hydrolytically to aminophenols which could be condensed with aromatic aldehydes to yield 2-substituted dihydro-1,3-oxazines. From hydroquinone, isomeric pairs of bis-dihydroöxazines were obtained and structures assigned on the basis of dipole measurements. The dihydro-*m*-oxazines as a class show inhibition of adenocarcinoma E0771. This activity is retained in the corresponding methiodides and aminophenols as well as non-benzenoid tetrahydro-*m*-oxazines.

Introduction.—The organic chemist's intensive efforts to synthesize compounds which will inhibit unrestricted neoplastic growth have led to effective agents which may be grouped into three categories: (a) alkylating agents, (b) antimetabolites and (c) diverse unrelated compounds which have been discovered either by random antitumor screening or because of a special therapeutic interest in some other

Abbreviations Used in Tables I–X.—Solvents: *a*, (methylene chloride-) methanol; *b*, (methylene chloride-) ethanol; *c*, (methylene chloride-) benzene; *d*, (methylene chloride-) ethyl acetate; *e*, (methylene chloride-) cyclohexane; *f*, (methylene chloride-) heptane; *g*, chloroform; *h*, methanol-ether; *i*, water. Substituent groups: *j*, 3-picolyl; *k*, 4-picolyl; *l*, 3,4-dimethoxyphenethyl; *m*, 2-picolyl; *p*, 4-pyridyl; *q*, 3-pyridyl; *r*, 2-pyridyl. Other: *n*, partial destruction on chromatography; *o*, 0.01 molar in benzene except 0.004 molar for no. 76, 77, 84, 85; *s*, orally active.

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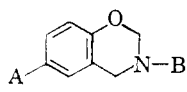
TABLE I



No.	A	B	Emp. formula	C Calcd.	C Found
1	CH_3	C_6H_{11}	$C_{15}H_{21}NO$	77.89	77.63
2	CH_3	$CH_2C_6H_5$	$C_{16}H_{17}NO$		Reported[a]
3	CH_3	$CH(CH_3)C_6H_5$	$C_{17}H_{19}NO$	80.60	80.32
4	CH_3	CH_2CONH_2	$C_{11}H_{14}N_2O_2$	64.06	63.80
5	Br	$CH_2C_6H_5$	$C_{15}H_{14}BrNO$	59.22	59.06
6	Br	CH_2CONH_2	$C_{10}H_{11}BrN_2O_2$	44.30	44.35
7	Br	(3)$CH_2C_5H_4N$[j]	$C_{14}H_{13}BrN_2O$	55.09	55.42
8	Br	(4)$CH_2C_5H_4N$[k]	$C_{14}H_{13}BrN_2O$	55.09	54.97
9	OCH_3	CH_3	$C_{10}H_{13}NO_2$	67.02	66.87
10	OCH_3	$CH_2C_6H_5$	$C_{16}H_{17}NO_2$	75.27	75.02
11	OCH_3	$CH_2CH_2C_6H_5$	$C_{17}H_{19}NO_2$	75.81	75.96
12	OCH_3	$CH_2CH_2C_6H_3(OCH_3)_2$[l]	$C_{19}H_{23}NO_4$	69.28	69.15
13	OCH_3	$CH_2CO_2CH_3$	$C_{12}H_{15}NO_4$	60.75	60.61
14	OCH_3	CH_2CONH_2	$C_{11}H_{14}N_2O_3$	59.45	59.56
15	OCH_3	(3)$CH_2C_5H_4N$[j]	$C_{15}H_{16}N_2O_2$	70.29	70.25
16	OCH_3	(4)$CH_2C_5H_4N$[k]	$C_{15}H_{16}N_2O_2$	70.29	69.94
17	$NHCOCH_3$	C_6H_{11}	$C_{16}H_{22}N_2O_2$	70.09	70.17
18	$NHCOCH_3$	$CH_2C_6H_5$	$C_{17}H_{18}N_2O_2$	72.32	72.24
19	$NHCOCH_3$	$CH_2CH_2C_6H_5$	$C_{18}H_{20}N_2O_2$	72.95	73.17
20	$NHCOCH_3$	$CH(CH_3)C_6H_5$	$C_{18}H_{20}N_2O_2$	72.95	73.13
21	$NHCOCH_3$	CH_2CONH_2	$C_{12}H_{15}N_3O_3$	57.82	57.50
22	$NHCOCH_3$	(2)$CH_2C_5H_4N$[m]	$C_{16}H_{17}N_3O_2$	67.82	67.37
23	$NHCOCH_3$	(3)$CH_2C_5H_4N$[j]	$C_{15}H_{17}N_3O_2$	67.82	67.49
24	$NHCOCH_3$	(3)$CH_2C_5H_4NCH_3^+ \cdot I^-$	$C_{17}H_{20}IN_3O_2$	48.01	47.50
25	$NHCOCH_3$	(4)$CH_2C_5H_4N$[k]	$C_{15}H_{17}N_3O_2$	67.82	67.52
26	$N(CH_3)COCH_3$	$CH_2C_6H_5$	$C_{18}H_{20}N_2O_2$	72.95	73.02
27	NO_2	$CH_2C_6H_5$	$C_{15}H_{14}N_2O_3$	66.65	66.69
28	NO_2	$CH_2CH_2C_6H_5$	$C_{15}H_{16}N_2O_3$	67.59	67.36
29	NO_2	$CH_2CH_2C_6H_3(OCH_3)_2$[k]	$C_{18}H_{20}N_2O_5$	62.78	63.05

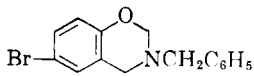
area. A survey of the last category was prompted by our anticipation of a synthetically exploitable common principle for some of its members, by which they might be related to either alkylating agents or antimetabolites.

Our attention centered upon the reported[2] inhibition of Crocker sarcoma in mice by three dihydrobenzoxazines (I, II, III). In order

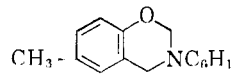
(2) T. Urbanski, Cz. Radzikowski, Z. Ledochowski and W. Czarnocki, *Nature*, **178**, 1351 (1956).

H		N			Carcinostatic activities		
					E0771 tumor during and after treatment		
Calcd.	Found	Calcd.	Found	M.p., °C.	7 day	1 day	Other tumors
9.15	9.02	6.05	5.95	38–39[a] rep. 30–32[g]	−/*	+/−	Leukemia 1210; Ehrlich ascites; sarcoma 180 (Subcut.) sl. inhib.
m.p. 71°				71–72[b]	++/**	++/*	
7.56	7.74	5.53	5.71	42–43[b]	−/−[s]	+/*	Ehrlich ascites sl. inhib.
6.84	6.92	13.58	13.67	182–183[b]		−/−	
4.63	4.83	4.60	4.61	80–81[a] rep. 85–87[g]		−/−	
4.09	4.17	10.34	10.24	187–188[b]	+/*		
4.30	4.35	9.18	9.40	99–100[f]		−/−	
4.30	4.22	9.18	9.21	111–112[a]		−/−	Sarcoma 180 sl. inhib.
7.31	7.27	7.82	7.87	118–119[d]	±/*	+/−	
6.71	6.76	5.49	5.52	74–75[a]		−/−	Ehrlich ascites sl. inhib.
7.11	6.99	5.20	5.08	73–74[a]	±/*	+/−	Ehrlich ascites, sarcoma 180 sl. inhib.
7.04	7.20	4.25	4.35	66–67[e]		−/−	
6.37	6.39	5.90	5.91	128 (0.001 mm.) liq.	−/−	−/*	Sarcoma 180 sl. inhib.
6.35	6.29	12.60	12.77	184–185[b]	+/*	+/−	Sarcoma 180 sl. inhib.
6.29	6.37	10.93	11.00	89–90[a]	−/−		
6.29	6.29	10.93	10.90	99–100[a]		−/−	Ehrlich ascites sl. inhib.
8.08	8.15	10.21	9.90	138–139[c]	+/*	++/*	
6.43	6.49	9.92	9.98	167–168[a,c] rep.[3] 168		−/−	
6.80	6.94	9.45	9.64	131–132[a]	−/−	+/*	
6.80	6.87	9.45	9.42	145–146[e]	+/**	+/*	
6.07	6.15	16.86	16.66	196–197[b]		−/−	
6.05	5.97	14.83	15.09	138–139[d]		−/−	
6.05	6.03	14.83	15.14	151–152[c]		+/**	Sarcoma 180 sl. inhib.
4.74	4.79	9.88	9.25	Amorph.[h]	+/*(*)		
6.05	6.22	14.83	14.66	176–177[a,c]		−/−	
6.80	6.85	9.45	9.16	83–84[e]		+/−	
5.22	5.41	10.37	10.44	88–89[a]	−/−		
5.67	5.74	9.85	9.82	84–85[a]	−/**		Ehrlich ascites sl. inhib. (subcut.)
5.85	5.93	8.14	8.45	77–78[e]	−/−		

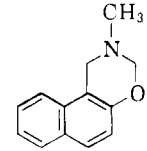
to ascertain whether compounds of this class generally produce some



I

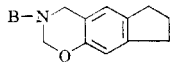


II

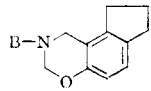


III

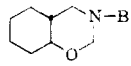
TABLE II



No.	B	Emp. formula	C Calcd.	C Found	H Calcd.	H Found
30	CH_3	$C_{12}H_{15}NO$	76.15	76.40	7.99	8.05
31	C_6H_{11}	$C_{17}H_{23}NO$	79.32	79.24	9.01	9.11
32	$CH_2C_6H_5$	$C_{18}H_{19}NO$	81.49	81.09	7.22	7.15
33	CH_2CONH_2	$C_{13}H_{16}N_2O_2$	67.22	67.45	6.94	7.04

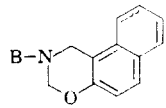


34	$CH_2C_6H_5$	$C_{18}H_{19}NO$	81.49	81.73	7.22	7.32



35	H	$C_8H_{15}NO$	...	...	...	...
36	p-$COC_6H_4NO_2$	$C_{15}H_{18}N_2O_4$	62.05	62.36	6.25	6.12
37	COC_6H_5	$C_{15}H_{19}NO_2$	73.74	73.47	7.81	7.88

TABLE III



No.	B	Emp. formula	C Calcd.	C Found	H Calcd.	H Found
38	CH_3	$C_{13}H_{13}NO$			Reported[5]	
39	$CH(CH_3)C_6H_5$	$C_{20}H_{19}NO$	83.00	83.07	6.62	6.74
40	$CH_2CH_2C_6H_3(OCH_3)_2$[i]	$C_{22}H_{23}NO_3$	75.62	75.82	6.63	6.64
41	$CH_2CO_2CH_3$	$C_{15}H_{15}NO_3$	70.02	69.81	5.88	5.85
42	CH_2CONH_2	$C_{14}H_{14}N_2O_2$	69.40	69.14	5.83	5.95
43	(2)$CH_2C_5H_4N$[m]	$C_{18}H_{16}N_2O$	78.23	78.30	5.84	5.88
44	(3)$CH_2C_4H_4N$[j]	$C_{18}H_{16}N_2O$	78.23	78.48	5.84	5.96
45	(4)$CH_2C_5H_4N$[k]	$C_{18}H_{16}N_2O$	78.23	78.48	5.84	5.96

carcinostasis, a large number of examples were synthesized and tested in experimental tumor systems. The widespread and tumor specific antineoplastic effect of dihydro-*m*-oxazines soon became apparent. Further structural variations were then aimed at a better understanding of the relationship between the characteristic labile N-C-O component of these compounds and biological activity.

Chemical Results.—The dihydroöxazines listed in Tables I to IV were obtained in extension of previous studies,[3-9] by condensation of phenols with primary amines and two equivalents of formaldehyde.

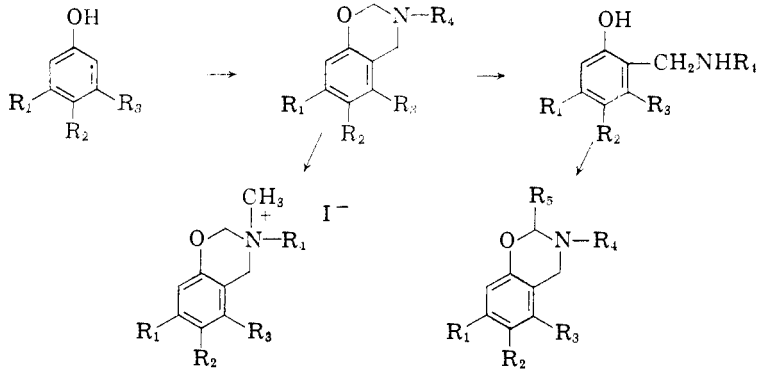
N			Carcinostatic activities		
			E0771 tumor during and after treatment		
Calcd.	Found	M.p., °C.	7 day	1 day	Other tumors
7.40	7.45	90–91[b]		−/*	Ehrlich ascites sl. inhib.
5.44	5.32	81–82[b]	++/*	±/*	Ehrlich ascites sl. inhib.
5.28	5.14	98–99[a]		−/−	Ehrlich ascites sl. inhib.
12.06	12.04	202–203[b]		−/−	
5.28	5.46	66–67[a]	+/*		
...	...	B.p. 150° (5 mm.)	+/**		Ehrlich ascites mol. inhib.
9.65	9.66	140–141[f]			
5.71	5.67	123–124[b]			

N			Carcinostatic activities		
			E0771 tumor during and after treatment		
Calcd.	Found	M.p., °C.	7 day	1 day	Other tumors
m.p. 67–68		67–68[a,f]		−/−	Ehrlich ascites, sarcoma 180 sl. inhib.
4.84	4.63	75–77[b]	++/**	[g]++/**	
4.01	3.82	87–88[a,e]		++/−	
5.44	5.35	93–94[a]		−/−	
11.56	11.87	202–203[b]		−/−	
10.14	9.61	84–85[a]		−/−	Sarcoma 180 sl. inhib.
10.14	10.15	79–80[a]		−/−	
10.14	10.21	92–93[b]		−/−	

In some instances, primarily with 2-aminomethylpyridine, the phenols reacted with amines in a ratio of 2:1 to give the condensation products of Table IX. Heating of the dihydro-*m*-oxazines with 3% sul-

(3) A. J. Burke, *J. Am. Chem. Soc.*, **71**, 609 (1949).
(4) A. J. Burke and C. Weatherbee, *ibid.*, **72**, 4691 (1950).
(5) A. J. Burke, R. P. Smith, and C. Weatherbee, *ibid.*, **74**, 602 (1952).
(6) A. J. Burke, M. J. Kolbezen, and C. W. Stephens, *ibid.*, **74**, 3601 (1952).
(7) A. J. Burke and R. J. Reynolds, *ibid.*, **76**, 1291 (1954).
(8) A. J. Burke, K. C. Murdock, and G. Ec, *ibid.*, **76**, 1677 (1954).
(9) T. Urbanski, D. Gürne, Z. Eckstein, and S. Slopek, *Bull. acad. polon. sci. Classe III*, **3**, 397 (1955).

furic acid or 5% potassium hydroxide solution liberated formaldehyde, which could be distilled and colorimetrically measured after reaction with chromotropic acid. Quaternization of dihydro-*m*-oxazines in methyl iodide, at room temperature, gave the methiodides. In contrast to their parent compounds, the quaternary salts listed in Table VI were stable to acid and base. Utilizing this resistance to hydrolytic cleavage, it was possible to locate the position of monoquaternization in dihydro-*m*-oxazines containing a second basic nitrogen. Thus it was found that *N*-3 and 4-picolyl dihydro-*m*-oxazines are quaternized preferentially on the pyridine nitrogen.



Methiodides with β-phenethyl substituents on nitrogen also liberated formaldehyde when treated with base (but not acid). Hofmann elimination of styrene and normal cleavage of the resulting *N*-methyl dihydro-*m*-oxazines can be expected in these exceptional cases. A facile Hofmann elimination also was observed on quaternization of the *N*-α-phenylethyldihydro-*m*-oxazine derived from β-naphthol. Refluxing the dihydroöxazine in a mixture of methanol and methyl iodide yielded only the *N*,*N*-dimethyl methiodide.

Preparative hydrolytic cleavage of the dihydro-*m*-oxazine ring with aqueous acid resulted in the aminophenols shown in Table VII. From condensation of these aminophenols with aromatic aldehydes or the three pyridine aldehydes, the methylene substituted dihydro-*m*-oxazines of Table VIII were obtained. A similar reaction with acetaldehyde could not be effected.

Structural assignments to the coumarin dihydro-*m*-oxazines were based on chemical degradations and n.m.r. spectral studies. Re-

versible hydrolysis of the *N*-dimethoxyphenethyl compound IV with methanolic hydrochloric acid, to the aminophenol V, and acetolysis to the diacetyl compounds VI and VII with acetic acid-acetic anhydride, substantiated the dihydro-*m*-oxazine structure. During hydrolysis a Pictet–Spengler formation of the isoquinoline VIII was observed as a side reaction.

The finding of two doublets in the aromatic hydrogen region of the n.m.r. spectra (see experimental) of compounds IX, X and further analogs and a singlet for the benzylic *N*-substituted methylene group

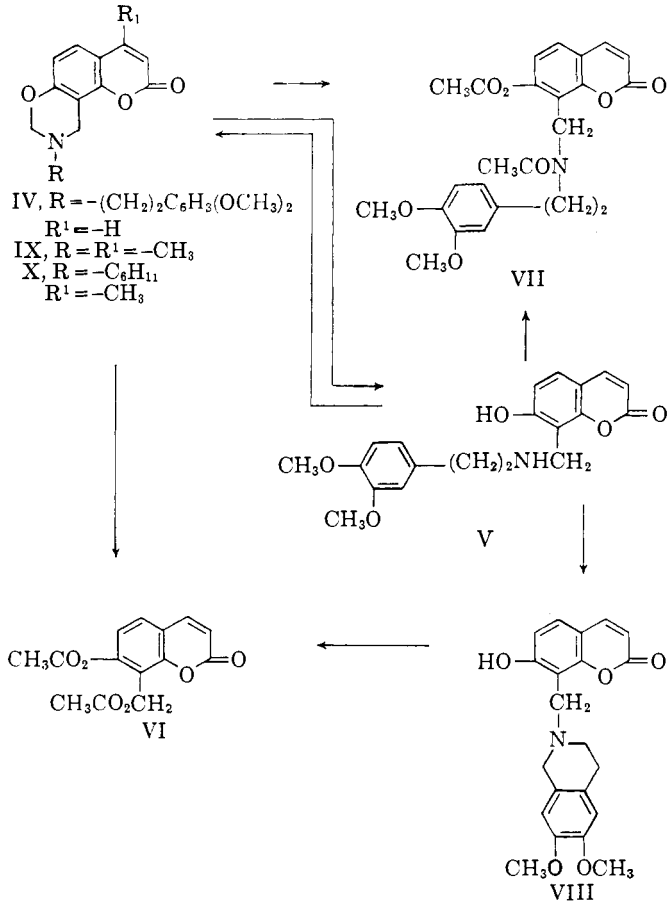
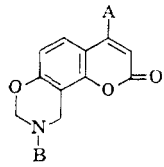


TABLE IV



No.	A	B	Emp. formula	C Calcd.	C Found	H Calcd.	H Found
46	CH_3	CH_3	$C_{13}H_{13}NO_3$	67.52	67.15	5.67	5.76
47	CH_3	C_6H_{11}	$C_{18}H_{21}NO_3$	72.20	72.33	7.07	7.15
48	CH_3	$CH_2C_6H_5$	$C_{19}H_{17}NO_3$	74.25	74.42	5.58	5.79
49	CH_3	$CH_2CH_2C_6H_5$	$C_{20}H_{19}NO_3$	74.74	74.25	5.96	5.87
50	CH_3	$CH(CH_3)C_6H_5$	$C_{20}H_{19}NO_3$	74.74	74.77	5.96	5.97
51	CH_3	$CH_2CH_2C_6H_3(OCH_3)_2$[l]	$C_{22}H_{23}NO_5$	69.27	68.90	6.08	5.87
52	CH_3	CH_2CONH_2	$C_{14}H_{14}N_2O_4$	61.31	60.82	5.15	5.10
53	CH_3	$CH_2CO_2CH_3$	$C_{15}H_{15}NO_5$	62.28	62.58	5.23	5.59
54	CH_3	(2) $CH_2C_5H_4N$[m]	$C_{18}H_{16}N_2O_3$	70.11	69.70	5.23	5.19
55	CH_3	(3) $CH_2C_5H_4N$[i]	$C_{18}H_{16}N_2O_3$	70.11	70.07	5.23	5.16
56	CH_3	(4) $CH_2C_5H_4N$[k]	$C_{18}H_{16}N_2O_3$	70.11	70.08	5.23	5.45
57	CH_3	(4) $CH_2C_4H_4N^+CH_3I^-$[k]	$C_{19}H_{19}IN_2O_3$	50.68	50.48	4.26	4.44
58	H	CH_3	$C_{12}H_{11}NO_3$	66.35	67.04	5.10	5.23
59	H	C_6H_{11}	$C_{17}H_{19}NO_3$	71.57	71.90	6.71	6.88
60	H	$CH_2C_6H_5$	$C_{18}H_{15}NO_3$	73.70	73.46	5.15	5.19
61	H	$CH_2CH_2C_6H_5$	$C_{19}H_{17}NO_3$	74.25	74.41	5.58	5.46
62	H	$CH_2CH_2C_6H_3(OCH_3)_2$[l]	$C_{21}H_{21}NO_5$	68.65	68.47	5.76	5.64
63	H	CH_2CONH_2	$C_{13}H_{12}N_2O_4$	59.99	60.00	4.65	4.87
64	H	(2) $CH_2C_5H_4N$[m]	$C_{17}H_{14}N_2O_3$	69.37	69.26	4.80	4.84
65	H	(3) $CH_2C_5H_4N$[i]	$C_{17}H_{14}N_2O_3$	69.37	69.54	4.80	5.02
66	H	(4) $CH_2C_5H_4N$[k]	$C_{17}H_{14}N_2O_3$	69.37	69.41	4.80	4.73

is consistent[10] with the assigned angular ring fusion of the oxazine and coumarin moieties (adjacent non-equivalent aromatic hydrogens) and excludes linearly fused structures.

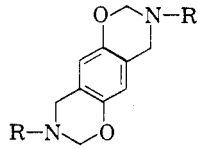
Two isomeric bis-dihydro-*m*-oxazine products, XI and XII, can be expected from the condensation of hydroquinone with formaldehyde and primary amines. A previous investigation[4] had yielded only one isomer when methylamine or cyclohexylamine was employed. The *anti* structure XI was established[4] for the methylamine product by its conversion to 2,5-bis-dimethylaminomethylhydroquinone, which had been related[11] to quinone-2,5-dicarboxylic acid. In our hands, examination of these and analogous reactions by chromatography of the crude products led to isolation of the *syn* isomers XII as the preponderant products from the reaction with methylamine as well as most other amines (Table V). Structural determinations within each isomer pair were based on dipole measurements. Assignment of

(10) L. M. Jackman in "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Ltd., London, England, 1959, p. 89.

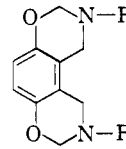
(11) W. T. Caldwell and T. R. Thompson, *J. Am. Chem. Soc.*, **61**, 765 (1939).

—N—			Carcinostatic activities		
			E0771 tumor during and after treatment		
Calcd.	Found	M.p., °C.	7 day	1 day	Other tumors
6.06	5.89	158–159[b]		−/−	
4.68	4.73	128–129[b]		−/−	
4.56	4.33	135–136[b]	−/−	+/*	
4.36	4.25	119–120[b]	+/−	+/*	
4.36	4.65	116–117[b]		−/−	
3.67	3.77	133–134[a,c]	−/−	+/−	
10.21	9.98	198–199[b]		−/−	Leukemia 1210 sl. inhib.
4.84	4.61	127–128[a]	−/−		
9.09	9.07	160–161[a]		−/−	
9.09	9.39	163–164[b]		−/−	
9.09	9.03	155–156[b,c]	−/−	+/*	
6.22	6.24	198–199[a]	++(+)/**(*)		
6.45	8.82	139–140[b]		−/−	
4.91	4.91	125–126[b]		−/−	
4.78	4.54	127–128[b]	−/−	+/*	Ehrlich ascites sl. inhib.
4.56	4.65	118–119[a]		−/−	Ehrlich ascites sl. inhib.
3.81	3.92	138–139[a,e]	+++/***[3]	+/*	Sarcoma 180, Ehrlich ascites sl. inhib.
10.77	10.82	194–195[b]		−/−	
9.52	9.77	144–145[a,c]		−/−	
9.52	9.40	137–138[b]	−/−		
9.52	9.37	143–144[c]	−/−	−/−	Ehrlich ascites sl. inhib.

syn structures to the compounds with higher capacitance values revealed a correlation with corresponding lower melting points.



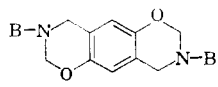
XI



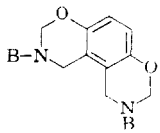
XII

Biological Methods.—Fragments of a ten-day, 2 mm. diameter, adenocarcinoma E0771 tumor were implanted by trocar subcutaneously into the right axillary region of C 57 B1 female mice. Seven days post implantation the recipients bearing a 5–7 mm. diameter tumor were selected and divided into groups of 10 mice each. Treatment was administered intraperitoneally at the maximum tolerated dose (100–250 mg./kg.) for ten consecutive days. A number of compounds were also tested by oral administration (s in Tables). Solid tumor diameters were measured with a vernier caliper and aver-

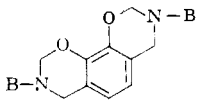
TABLE V



No.	B	Emp. formula	C Calcd.	C Found	H Calcd.	H Found
67	CH_3	$C_{12}H_{16}N_2O_2$				Reported[4]
68	C_6H_{11}	$C_{22}H_{32}N_2O_2$				Reported[4]
69	$CH_2C_6H_5$	$C_{24}H_{24}N_2O_2$	77.39	77.52	6.50	6.42
70	$CH_2CH_2C_6H_5$	$C_{26}H_{28}N_2O_2$	77.97	77.98	7.05	7.12
71	$CH(CH_3)C_6H_5$	$C_{26}H_{28}N_2O_2$	77.97	77.76	7.05	7.28
72	$CH_2CH_2C_6H_3(OCH_3)_2$[l]	$C_{30}H_{36}N_2O_6$	69.21	68.89	6.97	6.95
73	(3) $CH_2C_5H_4N$[j]	$C_{22}H_{22}N_4O_2$	70.57	70.10	5.92	5.96
74	(4) $CH_2C_5H_4N$[k]	$C_{22}H_{22}N_4O_2$	70.57	70.37	5.92	6.02
75	CH_2CH_2OH	$C_{14}H_{20}N_2O_4$	59.98	59.84	7.19	7.37



76	CH_3	$C_{12}H_{16}N_2O_2$	65.45	65.27	7.32	7.21
77	C_6H_{11}	$C_{22}H_{32}N_2O_2$	74.12	73.95	9.05	9.00
78	$CH_2C_6H_5$	$C_{24}H_{24}N_2O_2$	77.39	77.26	6.50	6.47
79	$CH_2CH_2C_6H_5$	$C_{26}H_{28}N_2O_2$	77.97	77.46	7.05	7.05
80	$CH_2CH_2C_6H_3(OCH_3)_2$[l]	$C_{30}H_{36}N_2O_6$	69.21	69.16	6.97	7.05
81	(3) $CH_2C_5H_4N$[j]	$C_{22}H_{22}N_4O_2$	70.57	70.73	5.92	6.11
82	(4) $CH_2C_5H_4N$[k]	$C_{22}H_{22}N_4O_2$	70.57	70.04	5.92	6.17
83	CH_2CH_2OH	$C_{14}H_{20}N_2O_4$	59.98	60.07	7.19	7.43



84	C_6H_{11}	$C_{22}H_{32}N_2O_2$				Reported[4]
85	$CH_2C_6H_5$	$C_{24}H_{24}N_2O_2$				Reported[4]
86	$CH(CH_3)C_6H_5$	$C_{26}H_{28}N_2O_2$	77.97	77.84	7.05	7.02

age sizes in treated groups and control groups compared on days ten (+ in Tables) and fifteen (five days post treatment, * in Tables). In preliminary studies with the E0771 tumor, treatment was initiated one day after tumor implanation and continued for 7 days. Here assessments of activities were made on the day following the last injection (+ in Tables) and after an additional observation period of 7 days (* in Tables). Evaluation: 0–24% inhibition = −; 25–49% = + or *; 50–74% = ++ or **; 75–100% = +++ or ***; ± or $\overset{*}{-}$ stand for variable activity. Values at end of treatment and

N		M.p., °C.	Yield, % (see Experimental)	Capacitance	Carcinostatic activities: E0771 tumor during and after treatment		Other tumors
Calcd.	Found				7 day	1 day	
m.p. 182–183°		183–184[d]	27	29643		−/−	Ehrlich ascites mod. inhib.
m.p. 161–162°		161–162[a]	41	29715		−/−	
7.52	7.39	203–204[c]	19	29564		−/−	Sarcoma 180 sl. inhib.
7.00	6.78	156–157[d]	10	29739		−/−	
7.00	7.09	173–174[d]	17	29749		−/−	
5.38	5.35	151–152[d]	8	29643		−/−	
14.96	14.92	203–204[d]	16	29795		−/−	
14.96	14.86	206–207[g]	2	29813	+/*		
9.99	9.96	110–111[d]	43	29566		−/−	Ehrlich ascites mod. inhib.
12.72	12.57	98–99[d,e]	64	29685		−/−	
7.86	7.68	87–88[a]	11	29877	++/**		
7.52	7.25	144–145[d]	80	29680	−/−	+/*	Ehrlich ascites sl. inhib.
7.00	6.89	102–103[b,d]	28	29778		−/−	
5.38	5.33	124–125[b,d]	11	29876		−/−	
14.96	14.65	127–128[d]	43	29824		−/−	
14.96	15.07	181–182[d]	22	29821		−/−	
9.99	9.70	158–159[d]	23	29843		−/−	Ehrlich ascites mod. inhib.
m.p. 143–144°		143–144[a]	14	...	+++/***[s] not active i.p.		
m.p. 182–183°		180–181[a]	17	...	++/*[s]		
7.00	6.76	225–226[c]	25	...	−/*		

post treatment observation periods are indicated by + and *, respectively.

All compounds also were tested against sarcoma 180 in CFW female mice and slight activity was found in a few cases. Testing of many of these compounds against lymphatic leukemia L 1210 in DBA/2 mice revealed slight activity in only two examples. A number of compounds exerted slight to moderate inhibition of Ehrlich's ascites tumor in CFW mice, but only when administered intraperitoneally 24 hours after injection of the tumor inoculum.

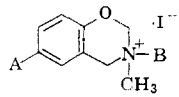
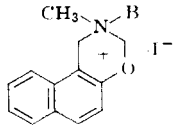
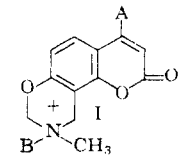


TABLE VI

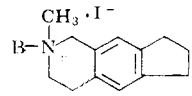
No.	A	B	Emp. formula	C Calcd.	C Found
87	OCH_3	$CH_2CH_2C_6H_5$	$C_{18}H_{22}INO_2$	52.57	52.80
88	$NHCOCH_3$	$CH(CH_3)C_6H_5$	$C_{19}H_{23}IN_2O_2$	...	...
89	$NHCOCH_3$	C_5H_{11}	$C_{17}H_{24}IN_2O_2$	49.04	49.36
90	$NHCOCH_3$	$CH_2CH_2C_6H_5$	$C_{19}H_{23}IN_2O_2$	...	...
91	$NHCOCH_3$	(3)$CH_2C_5H_4N^+CH_3\cdot I^-$	$C_{18}H_{23}I_2N_3O_2$	38.12	37.84



92		CH_3	$C_{14}H_{16}INO$	49.28	49.35
93		$CH(CH_3)C_6H_5$	$C_{21}H_{22}INO\cdot H_2O$	56.08	56.46
94		$CH_2CH_2C_6H_3(OCH_3)_2$[1]	$C_{23}H_{26}INO_3$	56.21	56.04



95	H	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	$C_{22}H_{24}INO_5\cdot CH_3OH$	51.03	50.82
96	CH_3	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	$C_{23}H_{26}INO_5$	52.78	53.08
97	CH_3	(4)$CH_2C_5H_4N^+CH_3I^-$	$C_{20}H_{22}IN_2O_3$	40.56	39.69



98		$CH_2C_6H_5$	$C_{19}H_{22}INO$	56.03	55.91

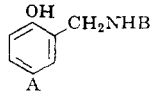
Acute toxicity studies indicated an LD_{50}, by intravenous injection, above 400 mg./kg. for the oxazines and minimum values of 250 mg./kg. for some quaternary compounds. A further investigation of the coumarin oxazine IV (orally active against E0771) showed no symp-

H		N		I		M.p., °C.	Carcinostatic Activities E0771 7 day tumor during and after treatment	Other tumors
Calcd.	Found	Calcd.	Found	Calcd.	Found			
5.39	5.61	3.41	3.50	...	...	160–161[a]	±/±	
...	...	...	...	...	...	Amorphous[h]	−/−	
6.05	6.30	6.73	6.78	...	...	233–234[a]	+++/***[s]	
...	...	...	...	...	...	Amorphous acetone–ether ppt.	++/**[s]	Ehrlich ascites. sl. inhib.
4.09	4.29	7.41	7.56	44.77	43.68	Amorphous[h]	++/**	
4.73	4.79	4.11	4.03	37.20	37.36	209–210[a]	+/**	Ehrlich ascites sl. inhib.
5.38	5.54	3.11	3.19	...	...	Amorphous aq. acetone–ether ppt.	++/**	Ehrlich ascites sl. inhib.
5.34	5.35	2.85	2.81	...	...	199–200[a]	−/−	
5.22	5.14	2.59	2.57	...	...	199–200[a]	++(+)/**(*)	
5.01	5.24	2.68	2.91	...	...	205–206[a]	++/**[s]	
3.75	3.76	4.73	5.04	43.88	44.41	248–249[a]	++[s]/***	
5.45	5.60	3.44	3.41	...	...	199–200[a]	+/*	Ehrlich ascites sl. inhib.

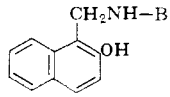
toms on oral administration of 250 mg./kg. to rats or mice for 30 days.

General Discussion.—The hydrolytic lability of di- and tetrahydro-*m*-oxazines suggested these compounds as non-specific, *in vivo*,

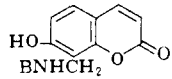
TABLE VII



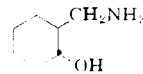
No.	A	B	Emp. formula	C Calcd.	C Found
99	OCH_3	$CH_2CH_2C_6H_5$	$C_{16}H_{19}NO_2$	74.68	74.30
100	OCH_3	$CH_2CH_2C_6H_5 \cdot HCl$	$C_{16}H_{19}NO_2 \cdot HCl$	65.40	65.40
101	OCH_3	CH_2CO_2H	$C_{10}H_{13}NO_4$	56.87	56.78
102	$NHCOCH_3$	C_6H_{11}	$C_{15}H_{22}N_2O_2$	68.68	68.48
103	$NHCOCH_3$	$C_6H_{11} \cdot HCl$	$C_{15}H_{22}N_2O_2 \cdot HCl \cdot H_2O$	56.87	57.27
104	$NHCOCH_3$	$CH_2CH_2C_6H_5$	$C_{17}H_{20}N_2O_2$	71.82	71.94
105	$NHCOCH_3$	$CH(CH_3)C_6H_5$	$C_{17}H_{20}N_2O_2$	71.82	72.02
106	$NHCOCH_3$	$CH(CH_3)C_6H_5 \cdot HCl$	$C_{17}H_{20}N_2O_2 \cdot HCl$	63.64	62.97



107		$CH(CH_3)C_6H_5 \cdot HCl$	$C_{19}H_{19}NO \cdot HCl$	72.72	72.80
108		CH_2CO_2H	$C_{13}H_{13}NO_3$	67.52	67.35



109	H	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	$C_{20}H_{21}NO_5$	67.58	67.38
110	H	$CH_2CH_2C_6H_3$-$(OCH_3)_2 \cdot HCl$	$C_{20}H_{22}NO_4 \cdot HCl$	61.31	61.34



111		$C_7H_{15}NO$		Reported[19]

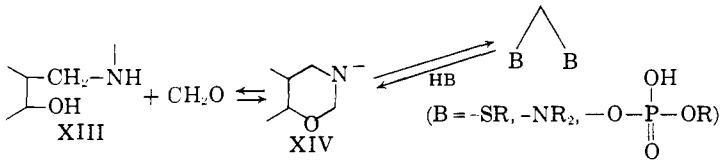
sources of formaldehyde or as non-specific methylene transfer compounds and thus as potential generators of the simplest possible bifunctional alkylating agent. Alternatively, di- and tetrahydro-*m*-oxazines could interfere specifically with the pathways involving one carbon metabolism. Thus formaldehyde and formate transfer by tetrahydrofolic acid[12,13] could find a competitive parallel in the reversible cleavage of di- and tetrahydro-*m*-oxazines (XIV), with the participating amino alcohols (XIII) serving in a corresponding formate transfer.

After confirming the antitumor activity of one of the reported three dihydroöxazines with a different test system (adenocarcinoma E0771

(12) M. J. Osborn, P. T. Talbert, and F. M. Huennekens, *J. Am. Chem. Soc.*, **82**, 4291 (1960).

(13) F. M. Huennekens, M. J. Osborn, and H. R. Whiteley, *Science*, **128**, 120 (1958).

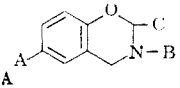
H		N			Carcinostatic activities E0771 tumor during and after treatment	
Calcd.	Found	Calcd.	Found	M.p., °C.	7 day	1 day
7.44	7.33	5.44	5.55	79–81[e]	−/−	
6.86	7.03	4.77	4.72	122–123[h]	++/**	
6.20	6.21	6.64	6.76	223–224[a]		−/−
8.45	8.50	10.68	10.54	163–164[a]	−/−	
7.95	8.23	8.84	8.82	156–157[i]	±/*	
7.09	7.05	9.85	9.56	148–149[c]	−/−	
7.09	7.12	9.85	9.30	145–146[c]	+/*	
6.60	6.80	8.74	8.98	133–135[i]	−/−	
6.42	6.52	4.46	4.39	158–160[h]		
5.67	5.63	6.06	5.91	232–234 (NaOH-NH_4Cl)	++/*	+/−
5.96	6.02	3.95	3.69	138–140[c]	−/−	
5.66	5.66	3.57	3.60	231–232[a]	+/**	
B.p. (15 mm.) 130–135				B.p. 135–140° (15 mm.)	−/**	Ehrlich ascites sl. inhib.



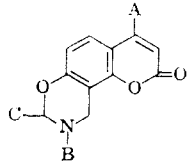
in mice), it was established that activity against this tumor is a general property of dihydro-*m*-oxazines by showing that 59 out of 148 compounds (Tables I–V, VIII, and following paper) has some inhibitory effect. In the process, a number of much more potent compounds were prepared and the examples IV, XII (R = cyclohexyl), and XV to XXIII selected as representatives with maximum activity, causing regression of the established tumor.

Frequency and spread of biological activity with variation of struc-

TABLE VIII



No.	A	B	C	Emp. formula
112	OCH_3	$CH_2CH_2C_6H_5$	p-C_6H_4Cl	$C_{23}H_{22}ClNO_2$
113	OCH_3	$CH_2CH_2C_6H_5$	p-$C_6H_4NO_2$	$C_{23}H_{22}N_2O_4$
114	$NHCOCH_3$	C_6H_{11}	C_6H_5	$C_{22}H_{26}N_2O_2$
115	$NHCOCH_3$	C_6H_{11}	o-C_6H_4Cl	$C_{22}H_{25}ClN_2O_2$
116	$NHCOCH_3$	C_6H_{11}	p-C_6H_4Cl	$C_{22}H_{25}ClN_2O_2$
117	$NHCOCH_3$	C_6H_{11}	p-$C_6H_4NO_2$	$C_{22}H_{25}N_3O_4$
118	$NHCOCH_3$	C_5H_{11}	(4) $C_5H_4N^p$	$C_{21}H_{25}N_3O_2 \cdot CH_3OH$
119	$NHCOCH_3$	C_6H_{11}	(3) $C_5H_4N^q$	$C_{21}H_{25}N_3O_2$
120	$NHCOCH_3$	C_6H_{11}	(2) $C_5H_4N^r$	$C_{21}H_{25}N_3O_2$
121	$NHCOCH_3$	$CH(CH_3)C_6H_5$	p-C_6H_4Cl	$C_{24}H_{23}ClN_2O_2$
122	$NHCOCH_3$	$CH(CH_3)C_6H_5$	p-$C_6H_4NO_2$	$C_{24}H_{23}N_3O_4$
123	$NHCOCH_3$	3-$CH_2C_6H_4N^j$	p-C_6H_4Cl	$C_{22}H_{20}ClN_3O_2$
124	$NHCOCH_3$	3-$CH_2C_4H_4N^j$	p-$C_6H_4NO_2$	$C_{22}H_{20}N_4O_4$

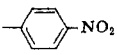


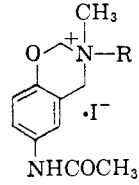
125	H	$CH_2CH_2C_6H_3(OCH_3)_2^l$	p-C_6H_4Cl	$C_{27}H_{24}ClNO_5$
126	H	$CH_2CH_2C_6H_3(OCH_3)_2^l$	p-$C_6H_4NO_2$	$C_{27}H_{24}N_2O_7$
127	H	$CH_2CH_2C_6H_3(OCH_3)_2^l$	4-$C_6H_4N^p$	$C_{26}H_{24}N_2O_5$

ture did not permit the assignment of optimum substitution on either the aromatic or heterocyclic moieties of the dihydrobenzoxazine system. Nor was any correlation of activity with basicity found[14]

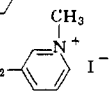
XV, R = -H

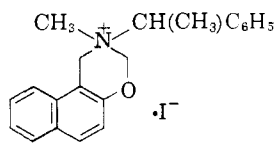
XVI, R = 

XVII, R = 



XVIII, R = 

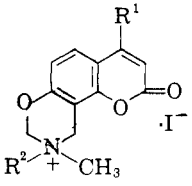
XIX, R = 

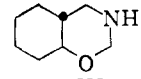


XX

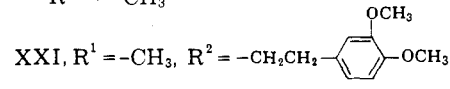
(14) Since relative and absolute pK values in a series of compounds depend on the solvent system, such measurements probably are illusory unless performed in physiological media.

C		H		N		M.p., °C.	Carcinostatic activities E0771 7 day tumor during and after treatment
Calcd.	Found	Calcd.	Found	Calcd.	Found		
72.71	72.39	5.84	5.95	3.69	3.57	78–79[a]	++/**
70.75	70.77	5.68	5.88	7.18	7.33	123–124[a]	+/*
75.40	75.23	7.48	7.66	7.99	7.89	173–175[f]	+/*
68.65	68.37	6.55	6.85	7.28	7.77	184–186[a]	+++/***
68.65	68.58	6.55	6.75	7.28	7.25	161–162[c]	+/−
66.82	66.81	6.37	6.48	10.63	10.57	104–107[b]	++/**
68.92	68.89	7.62	7.58	10.95	10.34	162–163[a]	+/−
71.77	71.25	7.17	7.25	11.96	11.82	208–209[a]	+++/−
71.77	71.28	7.17	7.16	11.96	11.90	193–194[a]	−/−
70.84	70.45	5.70	5.70	6.88	6.57	101–102[a]	+/−
69.05	68.88	5.55	5.65	10.07	10.21	163–164[a]	−/*
67.08	67.04	5.12	5.34	10.67	10.59	201–202[b]	−/−
65.34	65.17	4.99	5.07	13.86	14.00	205–206[b]	++/*
67.85	67.95	5.06	5.12	Cl, 7.42	7.29	104–105[a]	−/−
66.38	66.59	4.95	5.12	5.74	6.10	170–171[c]	−/*
70.25	70.18	5.44	5.54	6.30	6.42	157–159[a]	±/*

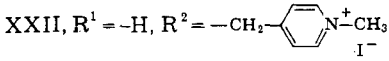




XXIII



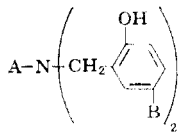
XXI, $R^1 = -CH_3$, $R^2 = -CH_2CH_2-$ (2-OCH_3, 4-OCH_3)



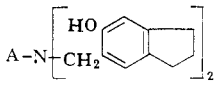
XXII, $R^1 = -H$, $R^2 = -CH_2-$ (4-N^+-CH_3 I^-)

(Table X). However, structural variation revealed four significant points: (a) substitution of the methylene group between oxygen and nitrogen with aryl and pyridyl groups can either destroy or increase the antitumor potency of an active dihydrobenzoxazine. Thus

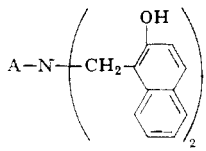
TABLE IX



No.	A	B	Emp. formula
128	CH_3	OCH_3	$C_{17}H_{21}NO_4$
129	CH_3 Dibenzoate	OCH_3	$C_{31}H_{29}NO_6$
130	2-$CH_2C_5H_4N^m$	CH_3	$C_{22}H_{24}N_2O_2$
131	2-$CH_2C_5H_4N^m$	OCH_3	$C_{22}H_{24}N_2O_4$
132	2-$CH_2C_5H_4N^m$	Br	$C_{20}H_{18}Br_2N_2O_2$
133	2-$CH_2C_5H_4N^m$	$NHCOCH_3$	$C_{24}H_{28}N_4O_4$
134	3-$CH_2C_5H_4N^j$	OCH_3	$C_{22}H_{24}N_2O_4$
135	3-$CH_2C_5H_4N^j$	$NHCOCH_3$	$C_{24}H_{28}N_4O_4$



136	2-$CH_2C_5H_4N^m$	$C_{26}H_{28}N_2O_2$



137	3-$CH_2C_5H_4N^j$	$C_{28}H_{34}N_2O_2$

activity is not related only to transfer of methylene or formaldehyde.[15] (In control studies, injection of formaldehyde, trioxymethylene or formaldehyde acetals had little effect on E0771 tumors but a 70% inhibition was found with hexamethylenetetramine). (b) Amino alcohols (XIII) derived from active dihydro-*m*-oxazines by hydrolytic cleavage, generally retain part of the antitumor activity of the parent compound. Biological activity of dihydro-*m*-oxazines may thus be due to the liberated amino alcohols, with the one carbon link of the oxazine providing stabilization and selective release in the tumor cell. (c) Quaternization of dihydrobenzoxazines increases stability to chemical hydrolysis. Antitumor activity is retained and frequently increased in these compounds, suggesting *in vivo* dequater-

(15) Biological activity of dihydrooxazines has been ascribed to the active —CH_2 group by T. Urbanski, D. Gürne, S. Slopek, H. Mordarska, and M. Mordarski, *Nature*, **187**, 426 (1960). The inhibition of Ehrlich ascites carcinoma and solid amobarbital ascites sarcoma by alicyclic tetrahydro-1,3-oxazines was shown.

C		H		N		M.p.,
Calcd.	Found	Calcd.	Found	Calcd.	Found	°C.
67.31	67.38	6.98	7.08	4.62	4.55	157–158[a]
72.78	72.59	5.71	5.73	...	...	101–102[a]
75.83	75.64	6.94	6.94	8.04	8.31	177–178[c]
69.45	69.60	6.36	6.47	7.36	6.98	150–151[a]
50.13	50.26	3.81	3.97	5.85	5.86	223–225[g]
66.03	65.88	6.47	6.38	12.84	12.46	162–164[a]
69.45	69.56	6.36	6.58	7.36	7.12	167–168[g]
66.03	65.88	6.47	6.38	12.84	12.46	164–165[b]
77.97	77.65	7.05	6.87	7.00	6.94	190–191[b,e]
79.97	79.95	5.75	5.74	6.66	6.40	161–162[b]

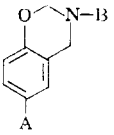
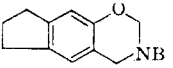
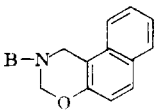
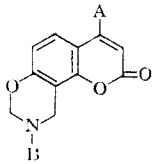
nization (analogous to the conversion of betaine to dimethylglycine). (d) Tumor inhibition was found with the alicyclic *m*-perhydroöxazine XXIII and the corresponding amino alcohol, demonstrating that an aromatic ring system is not essential.[15]

Acknowledgment.—Mr. L. Dorfman and his staff furnished microanalyses as well as frequent valuable help with analytical problems. Quantitative formaldehyde determinations were carried out by Dr. C. R. Rehm and Mr. L. Dorfman. We thank Dr. M. J. Allen for pK determinations and capacitance measurements, and Prof. H. Conroy and Prof. E. Wenkert for their valuable contributions in recording and interpreting n.m.r. spectra. Toxicity data were obtained by Dr. A. E. Earl and his associates. It is a pleasure to acknowledge the aid of Miss Olive Reynolds and Mr. Benjamin Lambert during the chemical work and the assistance of Mr. J. Tanzola and Mr. T. Gilgunn in the biological testing. Finally, we thank Dr. E. Schlittler for his valuable interest and support.

TABLE X

pKa' VALUES

Determined in 80% ethyleneglycol monomethyl ether at 20°

	No.	A	B	pKa'
	3	CH_3	$CH(CH_3)C_6H_5$	7.2
	4	CH_3	CH_2CONH_2	5.2
	5	Br	$CH_2C_6H_5$	5.1
	10	OCH_3	$CH_2C_6H_5$	5.4
	11	OCH_3	$CH_2CH_2C_6H_5$	6.2
	12	OCH_3	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	6.4
	14	OCH_3	CH_2CONH_2	4.7
	17	$NHCOCH_3$	C_6H_{11}	8.2
	18	$NHCOCH_3$	$CH_2C_6H_5$	5.5
	19	$NHCOCH_3$	$CH_2CH_2C_6H_5$	6.2
	20	$NHCOCH_3$	$CH(CH_3)C_6H_5$	7.0
	26	NCH_3COCH_3	$CH_2C_6H_5$	5.6
	27	NO_2	$CH_2C_6H_5$	5.3
	28	NO_2	$CH_2CH_2C_6H_5$	5.6
	32	...	$CH_2C_6H_5$	5.9
	39	...	$CH(CH)_3C_6H_5$	4.9
	40	...	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	5.4
	42	...	CH_2CONH_2	4.2
	48	CH_3	$CH_2C_6H_5$	4.9
	60	H	$CH_2C_6H_5$	4.8
	61	H	$CH_2CH_2C_6H_5$	6.4
	51	CH_3	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	6.5
	62	H	$CH_2CH_2C_6H_3(OCH_3)_2$[1]	5.9

Experimental[16]

General Procedure for Preparation of Dihydro-*m*-oxazines.—To a solution of 0.10 g. (0.0018 mole) of potassium hydroxide in 5 ml. of methanol was added 6.0 g. (0.066 mole) of trioxymethylene, the mixture warmed to effect solution and, with chilling in ice, 0.10 mole of the respective amine introduced, then 0.10 mole of the respective phenol and 20 ml. of methanol. After refluxing for 20 min. under nitrogen, the reaction mixtures were chilled, causing in some instances crystallization of the products. More generally, the viscous oils were dissolved in methylene chloride and the solutions washed well with water, dried over magnesium sulfate and concentrated in vacuum. The residues were dissolved in

(16) All melting points corrected.

benzene and petroleum ether was added to the point of cloudiness or, alternatively, methylene chloride added to effect complete solution. The solutions were then passed over a column of 50 g. of alumina (Woelm, basic, activity III) and the products eluted with benzene. (Solvents for recrystallizations are listed with the melting points of the compounds.) Yields ranged generally from 30–70% though in a few cases higher yields were obtained.

Notes: (1) Whenever methylamine was used, the reaction was conducted in aqueous methanol, employing 12.4 g. (0.10 mole) of 25% aqueous methylamine, 18.5 g. (0.23 mole) of 38% aqueous formaldehyde and 40 ml. of methanol with refluxing for 90 min. (2) *p*-Nitrophenol gave only traces of oxazines under the usual conditions but fair yields when the reactions were carried out in dioxane with aqueous formaldehyde. (3) *N*-Methyl-*p*-acetamidophenol was recovered unchanged after heating for 30 min., but fair yields of oxazines were obtained after refluxing for 6 hr. (4) In large scale preparations it was found advantageous to dissolve the phenolic compound first in a minimum amount of methanol.

Bis-dihydroöxazines.—The compounds were prepared according to the generalized procedure except for use of only 0.05 mole of *p*- or *o*-dihydroxybenzene with the same quantities of the other reagents. After concentration of the reaction mixtures obtained with hydroquinone, the higher melting isomer crystallized on trituration with methanol in all cases except the reaction with 4-aminomethylpyridine, where the lower melting isomer crystallized. With (a) benzylamine, (b) 3,4-dimethoxyphenethylamine, and (c) ethanolamine, a mixture of isomers was obtained and separated respectively by (a) difference in either solubility, (b) and (c) fractional crystallization from ethyl acetate. Chromatography of the mother liquor materials on basic alumina (activity II) in benzene–petroleum ether yielded the second crystalline isomer. Further chromatographic studies with the pure low melting (*syn*) isomer of the *N,N*-dicyclohexyl-bis-dihydroöxazine indicated that extensive decomposition of the material occurs under these conditions. Actual reaction yields of the *syn*-bisoxazines may thus be considerably higher than the isolated yields. Only one crystalline isomer was obtained in the reaction with α-phenylethylamine. Yields, melting points and recrystallization solvents are shown in Table IV with capacitance values which were determined on 0.01 molar benzene solutions except for two pairs where 0.004 molar solutions were used because of low solubility.

Hydrolytic Cleavage of Dihydroöxazines.—Dihydroöxazines derived from *p*-acetamidophenol or *p*-methoxyphenol were dissolved in methylene chloride, the solutions saturated with dry hydrogen chloride and the solvent evaporated in vacuum. The residual hydrochlorides were then dissolved in a minimum amount of water and a slow stream of nitrogen was passed through the solution for 4 days. (The hydrochloride of 4-acetamido-2-(*N*-cyclohexyl)-aminomethylphenol crystallized from solution as a hydrate in 24 hr.) After concentrating to dryness in vacuum, the residues were dissolved in methanol and again taken to dryness. Solution in a minimum of water and addition of sodium bicarbonate caused precipitation of the aminophenols which were recrystallized from the solvents indicated in Table VII. All aminophenols were recrystallized until a color reaction with chromotropic acid in 80% sulfuric acid was negative, indicating absence of traces of dihydroöxazines.

Acylation of 4-Acetamido-2-(*N*-cyclohexyl)-aminomethylphenol.—A solution of 1.0 g. of the aminophenol in 10 ml. of acetic anhydride was allowed to stand at room temperature for 48 hr., concentrated in vacuum and the residue triturated with ether. The insoluble material (1.0 g.), m.p. 113–118°, was recrystallized from methanol–ether, to give the phenolic diacetamide, m.p. 136–138°, lacking infrared absorption above 1700 cm.$^{-1}$ (ester C=O).

Anal. Calcd. for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20. Found: C, 66.78; H, 8.07; N, 8.86.

Concentration of the ether solution and recrystallization of the residue from methylene chloride–ethyl acetate yielded 0.15 g. of the triacetate m.p. 175–176°.

Anal. Calcd. for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.57; N, 8.09. Found: C, 66.03; H, 7.62; N, 8.24.

Reversible Hydrolytic Cleavage of 3-(3,4-Dimethoxyphenethyl)-3,4-dihydro-5-hydroxy-2H-1,3-benzoxazine-6-acrylic lactone (IV).—From a suspension of 11.0 g. of IV in 300 ml. of methanol and 40 ml. of 10% hydrochloric acid, most solvent was distilled off over 30 min. Fresh methanol was added, removed again by distillation and the hydrochloride of the aminophenol V precipitated from the concentrated solution by addition of ether. Crystallization from methanol yielded 12.0 g. of material, m.p. 222° The free base was obtained by stirring the powdered hydrochloride in 120 ml. of saturated sodium bicarbonate solution for 2 hr. Filtration, washing with a small amount of water and recrystallization from methylene chloride and benzene gave 10.0 g. of V, m.p. 138–139°. When the hydrolytic reaction mixture was refluxed for 45 min. without distillation of methanol, a crude hydrochloride mixture, m.p. 215–217°, was obtained, which yielded equal amounts of two products on treatment with sodium bicarbonate and fractional crystallization from methylene chloride and benzene. In addition to the aminophenol V, m.p. 138–139°, the tetrahydroisoquinoline VIII, m.p. 206–207°, was found.

Anal. Calcd. for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.82. Found: C, 68.64; H, 5.74; N, 3.88.

Refluxing a solution of 1.3 g. of the tetrahydroisoquinoline VIII in 50 ml. of methanol and 50 ml. of methylene chloride with 0.13 g. of trioxymethylene and a trace of potassium hydroxide for 4 hr., under nitrogen, led to complete recovery of the material. Under these conditions the aminophenol V was quantitatively converted to the dihydroöxazine IV, m.p. and mixed m.p. 137–138°; but mixed m.p. 115–125° with starting V.

Acetolysis of 3-(3,4-Dimethoxyphenethyl)-3,4-dihydro-5-hydroxy-2H-1,3-benzoxazine-6-acrylic Lactone (IV) and the Tetrahydroisoquinoline (VIII).—(a) A solution of 2.0 g. of IV in 10 ml. of acetic acid and 10 ml. of acetic anhydride was refluxed under nitrogen for 6 hr. Concentration in vacuum and trituration with ether yielded 0.6 g. of material, m.p. 152–155°. Repeated recrystallization from methylene chloride–methanol gave 0.3 g. of material, m.p. 171–175°, identical by infrared spectrum and mixed m.p. with the amide ester VII, obtained below from acylation of the aminophenol V. Concentration of the ether solution and recrystallization of the residue from cyclohexane, then from methanol, gave 0.6 g. of the diacetate VI, m.p. 124–125°.

Anal. Calcd. for $C_{14}H_{12}O_6$: C, 60.88; H, 4.38; $COCH_3$, 31.2. Found: C,

TABLE XI

NUCLEAR MAGNETIC RESONANCE SPECTRA[18,a]

Compound		N—CH₃		Ar—CH₂—N	O—CH₂—N
46 (IX)	7.61	7.43	...	5.88	5.17
47 (X)	7.64	...	7.35	5.76	4.95
59	...	...	7.29	5.76	4.97

Compound	Benzene ring		Pyran ring			
	H_A	H_B	H_α	H_β	JAB	$J_{\alpha\beta}$
46 (IX)	3.26	2.63	3.92	...	9.0	...
47 (X)	3.15	2.50	3.90	...	8.9	...
59	3.33	2.77	3.78	2.38	8.6	9.5

[a] Chemical shifts (in τ) and spin coupling constants (in c.p.s.) obtained in deuteriochloroform at 60 mc./sec. with tetramethylsilane as internal standard.

61.27; H, 4.41; $COCH_3$, 31.5 (The O-acetyl determination was done by chromic acid oxidation and by hydrolysis, the latter method giving a value of 33.8%).

(b) A solution of 0.2 g. of the tetrahydroisoquinoline VIII in 10 ml. of acetic anhydride and 10 ml. of acetic acid was refluxed for 6 hr. under nitrogen. After evaporation to dryness, 0.08 g. of crystalline material was obtained from methanol, m.p. and mixed m.p. with the analytical sample of the diacetate VI 124–125°. Mother liquor material, m.p. 81–96°, had infrared and ultraviolet spectral characteristics consistent with the expected *N*-acetyl-6,7-dimethoxytetrahydroisoquinoline, although a pure sample, m.p. 104–105°,[17] could not be obtained by fractional crystallization or sublimation.

(c) Acylation of 1.0 g. of the aminophenol V in 10 ml. of acetic anhydride at room temperature for 4 days and trituration of the concentrated reaction mixture gave 1.1 g. of material, m.p. 145–150°. Repeated recrystallization from methylene chloride–benzene yielded 0.4 g. of amide ester VII, m.p. 179–180°.

Anal. Calcd. for $C_{24}H_{25}NO_7$: C, 65.59; H, 5.73; N, 3.19; $COCH_3$, 19.6. Found: C, 65.93; H, 5.73; N, 3.17; $COCH_3$, 19.7.

2-Substituted Dihydroöxazines.—The compounds of Table VIII were obtained by refluxing equivalent amounts of the aminophenols of Table VII and the respective aromatic or heterocyclic aldehyde in methanol for 15 hr. under nitrogen. With benzaldehyde best results were obtained by using 2 equivalents of aldehyde; with *p*-chlorobenzaldehyde the use of dioxane and anhydrous calcium sulfate gave better yields. The products were obtained in 50–90% yield on concentration and recrystallization from the solvents indicated in Table VIII.

2-[(*N*-Carboxymethyl)-aminomethyl]-4-methoxyphenol.—To a solution of 6.0 g. of trioxymethylene (0.067 mole) and 0.1 g. of potassium hydroxide in 8 ml. of methanol was added 12.4 g. of *p*-methoxyphenol (0.10 mole), then a solution of 7.5 g. of glycine (0.10 mole) and 5.5 g. of potassium hydroxide (0.10 mole) in 25

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(18) G. V. D. Tiers, *J. Phys. Chem.* **62**, 1151 (1958).

ml. of methanol. After refluxing under nitrogen for 20 min., 50 ml. of methylene chloride was added to the cooled reaction mixture and the precipitated crude product recrystallized from aqeuous methanol, yielding 3.5 g. of substance, m.p. 223–224° (chromotropic acid test negative).

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.87; H, 6.20; N, 6.64. Found: C, 56.78; H, 6.21; N, 6.76.

α-[(*N*-Carboxymethyl)-aminomethyl]-β-naphthol.—A solution of 1.0 g. of 1,2-dihydro-2-carbomethoxymethyl-3H-naph[1,2e]-*m*-oxazine and 0.22 g. of potassium hydroxide in 10 ml. of methanol and 2 ml. of water was refluxed for 4 hr. under nitrogen. On addition of 0.22 g. of ammonium chloride, 0.80 g. of material crystallized from the cooled solution, m.p. 232–234°. The amphoteric material was recrystallized best by addition of ammonium chloride to a solution in dilute sodium hydroxide (chromotropic acid test negative).

Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.35; H, 5.63; N, 5.91.

***trans*-Octahydro-1,3-benzoxazine.**—A solution of 1.2 g. (0.013 mole) of trioxymethylene and a trace of potassium hydroxide in 8 ml. of ethanol was added to a solution of 5.2 g. (0.040 mole) of *trans*-2-aminomethylcyclohexanol[19] in 20 ml. of ethanol. After refluxing under nitrogen for 3 hr., the solvent was removed in vacuum and the residual viscous oil distilled from an oil jacketed flask at bath temperature 150° (5 mm.). A benzoate was made in dry pyridine and a *p*-nitrobenzoate in dry benzene (Table II).

Methiodides of Dihydroöxazines.—To 6.0 g. of a dihydroöxazine dissolved in a minimum amount of methylene chloride, 10 ml. of methyl iodide was added and the solution stored for 3 days under nitrogen. Quantitative yields of the methiodides were obtained on concentration in vacuum and recrystallization from methanol, or precipitation of amorphous products from methanol by addition of ether. From the *N*-pyridylmethyl substituted dihydroöxazines, bis-methiodides were obtained by refluxing the dihydroöxazine in 75 ml. of methanol and 15 ml. of methyl iodide for 48 hr. Using the latter procedure with the dihydroöxazine derived from β-naphthol and α-phenylethylamine resulted in Hofmann elimination and dimethylation to give a methiodide identical with the one obtained from the *N*-methyl dihydroöxazine.

Analytical Determination of Formaldehyde.—A mixture of 11–16 mg. of dihydro-*m*-oxazine or its methiodide and 4 ml. of 3% sulfuric acid or 5% potassium hydroxide was heated at 110–140°. Steam was passed through the reaction mixture and 95 ml. of condensed distillate collected and diluted to 100 ml. Of this, a 2-ml. aliquot was heated at 100° for 1 hr. with 10 ml. of concd. sulfuric acid and 100 mg. of disodium chromotropate.[20] Colorimetric comparison with standard solutions gave these yields of formaldehyde for the respective compounds: 17, acid, 76%; 24, acid, 74%, base, 72%; 39, acid, 61%; 51, base, 82%; 56, base, 67%; 57, acid, 96%; 87, base, 80%; acid, 0%; 89, acid, 0%, base 0%; 92, acid, 0%, base, 0%; 95, base, 40%, acid, 0%.

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