2H-1,4-Benzoxazin-2-ones

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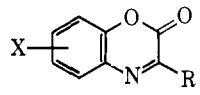
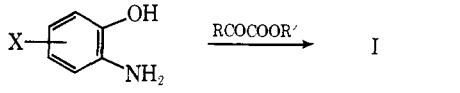
Thirty new 2H-1,4-benzoxazin-2-ones (I) were prepared by the reaction of *o*-aminophenols with α-keto esters. Wide pharmacological testing disclosed some interesting activities but little that was general for the series.

2H-1,4-Benzoxazin-2-ones (I) may be considered as 4-azacoumarins.



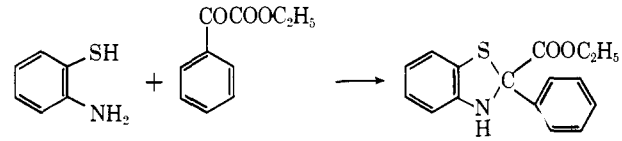
In 1897, Wislicenus and co-workers[1] reported this ring system as resulting from the condensation of α-keto esters with *o*-aminophenols. Later Biekert, *et al.*,[2] prepared a considerable number of such compounds; but except for a passing reference to bacteriological testing, no mention was made of biological properties.

Our interest in the chemistry of nitrogen-containing coumarins[3] and our desire to test these compounds for a variety of biological effects prompted us to prepare a number of 2H-1,4-benzoxazin-2-ones. These are listed in Table I. The method used for this preparation was essentially that of Wislicenus[1] and of Biekert.[2] However, no single procedure was found applicable in



all cases. The more reactive combinations of *o*-aminophenol and α-keto esters reacted at room temperature and the product needed only recrystallization. Less reactive combinations required heating at various temperatures up to 175°. Some of the products were difficult to purify, requiring sublimation and repeated crystallization. Most of the reactions were run only once so the yields may be far from optimum. However, the preparation of 3-methyl-2H-1,4-benzoxazin-2-one (**1**) was more extensively investigated. Whereas methyl pyruvate reacted at room temperature with *o*-aminophenol to give the desired compound in excellent yield, ethyl pyruvate, under identical conditions, gave only a small amount of a compound of unknown structure. At higher temperatures, ethyl pyruvate gave a poor yield of **1**. Some other ethyl α-keto esters gave good yields of benzoxazinones but, since the corresponding methyl esters were not available, it is not known whether these would have given still better yields.

An attempt was made to extend the reaction to *o*-aminobenzenethiol. However, with ethyl phenylglyoxylate, only the benzothiazoline II was obtained.



II

Pharmacology.—These compounds were subjected to a wide variety of pharmacological tests. They proved suprisingly nontoxic. Except as noted below, all had mouse intraperitoneal LD_{50} values[4] greater than 1000 mg/kg. The simplest member of the series, **1** (Table I), had LD_{50} = 650 mg/kg, **30** had LD_{50} = 422, and some of those containing phenolic hydroxyl groups were more toxic: **3**, 650; **4**, 200; and **10**, 300.

Except for CNS depression at high doses, there seems to be no activity general for the series. However, the following results seem worthy of mention. Compound **4** (Table I) inhibited O-methyltransferase *in vitro*[5] 100% at 10^{-3} *M* (I_{50} = 3 × 10^{-5} *M*), and **10** inhibited this enzyme 97% at 10^{-3} *M* (I_{50} = 2 × 10^{-5} *M*). Compound **4** inhibited 5-hydroxytryptophan decarboxylase *in vitro*[6] 88% at 10^{-2} *M* (I_{50} = 2 × 10^{-3} *M*); **28** protected mice against lethal doses of epinephrine 100% at 200 mg/kg, 67% at 100 mg/kg, and 33% at 50 mg/kg. The following compounds caused a decrease in motor activity[7] when given intraperitoneally to mice: **6**, 74% decrease at 200 mg/kg; 8, 68% at 200 mg/kg; **10**, 48% at 60 mg/kg; and **11**, 74% at 200 mg/kg. Three compounds were effective in the "fighting mouse" test[8] when administered intraperitoneally in low doses: **12**, ED_{50} (dose effective in 50% of the mice) = 7 mg/kg; **13**, ED_{50} = 9 mg/kg; and **25**, ED_{50} = 7 mg/kg.

Compound **22** was found to inhibit contractions of guinea pig ileum produced by slow reacting substance A (SRS-A).[9] It gave 25–50% inhibition at 6 μg/3 ml.

Compounds **12–14**, **21**, **25**, and **30** gave indication of antitumor activity as shown by cytotoxicity toward KB cells in agar by zone inhibition and/or by tube dilution.[10]

Compounds 8, **17**, **25**, **26**, **28**, and **31** were found to cause a reduction in excess of 30% (relative to controls)

(1) W. Wislicenus and W. Beckh, *Ann. Chem.*, **295**, 339 (1897); W. Wislicenus and F. Schultz, *ibid.*, **436**, 55 (1924); W. Wislicenus and E. Mundinger, *ibid.*, **436**, 62 (1924); W. Wislicenus and H. Bubeck, *ibid.*, **436**, 113 (1924).

(2) E. Biekert, D. Hoffmann, and F. J. Meyer, *Ber.*, **94**, 1664, 1676 (1961).

(3) R. B. Moffett, *J. Med. Pharm. Chem.*, **5**, 335 (1962); **7**, 446 (1964).

(4) For methodology see: R. B. Moffett, A. R. Hanze, and P. H. Seay, *ibid.*, **7**, 178 (1964).

(5) J. Axelrod, *Science*, **126**, 400 (1957).

(6) C. T. Clark, A. Weissbach, and S. Udenfriend, *J. Biol. Chem.*, **210**, 139 (1954).

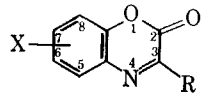
(7) R. B. Moffett and P. H. Seay, *J. Med. Pharm. Chem.*, **2**, 229 (1960).

(8) G. A. Youngdale, D. G. Anger, W. C. Anthony, J. P. DaVanzo, M. E. Greig, R. V. Heinzelman, H. H. Keasling, and J. Szmuszkovicz, *ibid.*, **7**, 415 (1964).

(9) Method of W. E. Brocklehurst, Ciba Foundation Symposium on Histamine, C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1956, p 175.

(10) C. G. Smith, W. L. Lummis, and J. E. Grady, *Cancer Res.*, **19**, 847 (1959).

TABLE: I 2H-1,4-BENZOXAZIN-2-ONES



No.	R	X	Moles of amino-phenols	Moles of α-keto esters[a]	Reaction temp, °C[b]	Reaction time,[c] hr	Yield, %[c]	Mp, °C	Crystn solvent	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found	Other, % Calcd	Other, % Found
1	CH_3	H	0.1	0.2	25	72	70[d]	101–103	95% EtOH	$C_9H_7NO_2$	67.07	67.17	4.38	4.56	8.69	8.44		
2	CH_3	6-Cl	0.1	0.2	120	1.25	12[e]	143.5–146.5	*i*-PrOH	$C_9H_7ClNO_2$	55.26	54.91	3.09	3.04	7.16	7.28	Cl, 18.13	Cl, 18.18
3	CH_3	5-OH	0.17[f]	0.34	100	0.083	28	135.5–137	*i*-PrOH	$C_9H_7NO_3$	61.01	60.89	3.98	3.98	7.91	8.06	O, 27.09	O, 27.23
4	CH_3	7,8-$(OH)_2$	0.03[g]	0.06[h]	100	*i*	42	249–252	EtOAc	$C_9H_7NO_4$	55.96	55.83	3.65	3.72	7.25	7.40	O, 33.13	O, 33.85
5	CH_3	5-CH=CHCH=CH-6	0.2[j]	0.34	25	*k*	8.6	161.5–163	*i*-PrOH	$C_{13}H_9NO_2$	73.93	73.77	4.29	4.31	6.63	6.53		
6	CH_3	6-C(CH$_3$)H=CHCOO-7,8-CH_3	0.1[l]	0.2[k]	25[m]	5	96	260–264	DMF[n]	$C_{14}H_{11}NO_4$	65.36	65.04	4.31	4.72	5.45	5.54		
7	$CH_2COOC_2H_5$	6-NO_2	0.1	0.2[o]	100	1	81	154–155	EtOH	$C_{12}H_{10}N_2O_6$	51.80	51.76	3.62	4.18	10.07	10.04		
8	$CH_2COOC_2H_5$	7-NO_2	0.1	0.2[o]	115	0.667	67	190–192	DMF[n]	$C_{12}H_{10}N_2O_6$	51.80	51.99	3.62	3.93	10.07	10.53	O, 34.51	O, 34.91
9	$CH_2COOC_2H_5$	7-OCH_3	0.1[p]	0.1	140	1	*p*	96.5–99	EtOH	$C_{13}H_{13}NO_5$	59.31	59.62	4.98	4.81	5.32	5.27		
10	$CH_2COOC_2H_5$	7,8-$(OH)_2$	0.06[q]	0.12	25	2.5[r]	84	156–158.5	EtOAc	$C_{12}H_{11}NO_6$	54.34	54.43	4.18	4.18	5.28	5.46		
11	$CH_2COOC_2H_5$	6-C(CH$_3$)H=CHCOO-7,8-CH_3	0.1[l]	0.2[o]	100	1	75	240–242.5	DMF[n]	$C_{17}H_{15}NO_6$	62.00	62.07	4.59	4.55	4.25	4.60	O, 29.15	O, 28.97
12	$CH(CH_3)COOC_2H_5$	7-NO_2	0.1	0.15	130	2.5	46	159.5–161	EtOH	$C_{13}H_{12}N_2O_6$	53.42	53.63	4.14	4.21	9.59	9.56		
13	$CH(CH_3)COOC_2H_5$	6-NO_2, 8-Cl	0.1[s]	0.3	160	3.5	18	130–132	EtOH	$C_{13}H_{11}ClN_2O_6$	47.79	47.65	3.39	3.75	8.58	8.40	Cl, 10.85	Cl, 10.87
14	—CHCH$_2$CH$_2$CH$_2$CO—	H	0.1	0.1	100	0.25	60	174–175	BuOH	$C_{13}H_{11}NO_3$	68.11	68.01	4.84	4.41	6.11	6.39		
15	—CHCH$_2$CH$_2$CH$_2$CO—	7-NO_2	0.1	0.1	130	1[t]	32	241–245	DMF[n]	$C_{13}H_{10}N_2O_5$	56.93	57.02	3.68	3.52	10.22	10.15		
16	—CHCH$_2$CH$_2$CH$_2$CO—	6,8-$(Cl)_2$	0.1	0.1	80	0.25[u]	48	206.5–208.5	MeOEtOH[v]	$C_{13}H_9Cl_2NO_3$	52.37	52.78	3.04	3.26	4.70	4.96	Cl, 23.78	Cl, 23.57
17	C_6H_5	6-Cl	0.2	0.4	140	2	86[w]	144–145.5	DMF[n]	$C_{14}H_8ClNO_2$	65.25	65.01	3.13	3.06	5.44	5.32	Cl, 13.76	Cl, 13.78
18	C_6H_5	6-NO_2	0.2	0.3	140	0.5	94	180–182	MeEtCO	$C_{14}H_8N_2O_4$	62.69	62.92	3.01	3.26	10.46	10.38		
19	C_6H_5	7-NO_2	0.1	0.2	140	1	49	140–142	EtOH	$C_{14}H_8N_2O_4$	62.69	62.46	3.01	3.14	10.46	10.78	O, 23.86	O, 24.44
20	C_6H_5	5-OH	0.125[x]	0.2	130	0.167[x]	42	183.5–184	Me_2CO–EtOH	$C_{14}N_9HO_3$	70.29	70.31	3.79	3.88	5.86	5.86	O, 20.07	O, 20.57
21	C_6H_5	7-OCH_3	0.134	0.1	100	1	15[e]	130–131.5	EtOH	$C_{15}H_{11}NO_3$	71.14	71.31	4.38	4.48	5.53	5.46		
22	C_6H_5	6-$SO_3^-NH_3^+$	0.05	0.1	150	2	56[e]	292.5–294.5 dec	EtOH	$C_{13}H_{12}N_2O_5S$	52.50	52.50	3.78	3.98	8.75	8.67	S, 10.01	S, 9.87
23	C_6H_5	5-CH=CHCH=CH-6	0.2[j]	0.4	140	0.5	80	177–178.5	MeEtCO	$C_{18}H_{11}NO_2$	79.11	78.87	4.06	4.10	5.13	5.20		
24	C_6H_5	6-C(CH$_3$)H CHCOO-7,8-CH_3	0.1[l]	0.1	175	2.5[y]	37	255–256	DMF[n]	$C_{19}H_{13}NO_4$	71.47	71.21	4.10	4.07	4.39	4.34		
25	3,4-$(OCH_3)_2C_6H_3$	H	0.08	0.06	150	1	94	160.5–161.5	DMF[n]–EtOH	$C_{16}H_{13}NO_4$	67.84	67.45	4.62	4.55	4.95	4.82		
26	3,4-$(OCH_3)_2C_6H_3$	7-NO_2	0.08	0.06	150	1.5[z]	57	200–202	MeOEtOH[v]	$C_{16}H_{12}N_2O_6$	58.54	58.63	3.68	3.73	8.54	8.65		
27	3,4-$(OCH_3)_2C_6H_3$	6-NO_2, 8-Cl	0.06[s]	0.05	150	2	100	259–261	DMF[n]	$C_{16}H_{11}ClN_2O_6$	52.98	52.96	3.06	3.22	7.72	7.55	Cl, 9.76	Cl, 9.70
28	3,4-$(OCH_3)_2)C_6H_3$	7,8-$(OH)_2$	0.06[q]	0.06	50	*aa*	61	240–241	Me_2CO	$C_{16}H_{13}NO_6$	60.95	61.04	4.16	3.85	4.44	4.53	O, 30.45	O, 30.05
29	4-Pyridyl	H	0.0254	0.0254	100	0.33[p]	38	168–169	EtOH	$C_{13}H_8N_2O_2$	69.64	69.72	3.59	3.49	12.50	12.82		
30	3-Indolyl	H	0.1	0.1	170	2	33	246.5–247	EtOH	$C_{16}H_{10}N_2O_2$	73.27	73.33	3.84	3.94	10.68	10.94		
31	3-Indolyl	6-CH_3	0.13	0.1	150	1.5	58	254–255.5	MeOEtOH[v]	$C_{17}H_{12}N_2O_2$	73.90	73.82	4.38	4.50	10.14	10.08		

[a] Unless otherwise indicated, ethyl esters were used. [b] Bath temperature. In those reactions run at 100° or higher, water and alcohol evaporated from the open flask. [c] Unless otherwise indicated, the reactions were run by the general procedure outlined in the Experimental Section. The yield is given for material melting not more than 2° below the highest melting point obtained. [d] Reported by Bickert, *et al.*,[2] in 50% yield using ethyl pyruvate, mp 98–99°. The preparation of this compound was investigated in some detail and is given in the Experimental Section. [e] The brown gummy reaction mixture was extracted with two portions of hexane at the boiling point and the remaining gum was sublimed at bath temperatures up to 175° (0.02 mm). About 5 g of crude material was obtained. Crystallization from 95% ethanol yielded 1.62 g of brown crystals, mp 135–140°. This was sublimed again at 125° (0.02 mm) and recrystallized from 2-propanol giving bright yellow crystals, mp 143.5–146.5°. [f] Free base was liberated by $NaHCO_3$ from 32.3 g (0.2 mole) of 2-aminoresorcinol hydrochloride and extracted with ether. The ether was evaporated below 30° under reduced pressure giving 21.4 g (0.17 mole) of brown solid. [g] 4-Aminopyrogallol was prepared as described in the Experimental Section from this quantity of 4-nitropyrogallol. [h] Methyl pyruvate was used. [i] The ethanol solution of the reactants was evaporated *in vacuo* on a steam bath giving a dark residue which was boiled with ethyl acetate, filtered, concentrated, and cooled giving greenish gray solid, mp 246–247°. By repeated recrystallization from ethyl acetate, with the aid of Darco G-60, 2.42 g of yellow crystals were obtained. [j] 1-Amino-2-naphthol was liberated from 0.2 mole of its hydrochloride. The solid was collected, dried, and used without further purification. [k] On standing at room temperature crystals separated from the reaction mixture which was mixed with ethanol and filtered. The solid was dissolved in benzene, filtered, and diluted with methanol giving yellow solid, mp 155–156°. The infrared spectrum and analysis indicates this is not the desired compound but may be an intermediate. The filtrates and solids were recombined, evaporated, and recrystallized from acetic acid giving greenish leaflets, which were recrystallized from isopropyl alcohol yielding 4.68 g (8.6%) of light yellow crystals, mp 161.5–163°. Infrared spectrum and analysis indicates this is the desired compound. [l] Prepared from 6-amino-7-hydroxy-4,8-dimethylcoumarin.[3] [m] At room temperature the reaction mixture became more fluid and then set solid. After warming on a steam bath for 1 hr the mixture was boiled with 85 ml of 95% ethanol and cooled. The solid was collected and dried giving 23.8 g of bright yellow solid, mp 260–264°. Recrystallization from DMF did not raise the melting point. [n] Dimethylformamide (DMF). [o] Diethyl oxalacetate was liberated from its sodium salt with concentrated HCl and extracted with ether. After drying (Na_2SO_4) the ether was removed and the oil was used without further purification. [p] Preparation described in the Experimental Section. [q] A solution of 4-aminopyrogallol hydrochloride was prepared as described in the Experimental Section from 10.27 g (0.06 mole) of 4-nitropyrogallol. The reaction mixture in ethanol was shaken under nitrogen for 2.5 hr, heated to the boiling point, and concentrated under reduced pressure. The residue was shaken with water and extracted with ether. [r] The ether solution was washed with water and dried (Na_2SO_4). After filtration the ether was evaporated giving 25.6 g. of light brown oil which soon crystallized. The solid was boiled with benzene, cooled, and filtered giving 13.3 g of yellow solid, mp 155–157°. [s] 2-Amino-6-chloro-4-nitrophenol hydrate was used. [t] The reactants were mixed in ethanol solution and evaporated to dryness before the heating. The crude product was sublimed in a bath up to 130° at 0.001 mm giving 9.4 g of orange solid. Recrystallization from DMF yielded 8.98 g of dark greenish crystals, mp 241–245°. [u] The reaction was run in ethanol solution. [v] 2-Methoxyethanol (Methyl Cellosolve). [w] The crude product was sublimed in a bath up to 170° (0.02 mm) giving 47.1 g of solid, which was recrystallized from a mixture of DMF and methanol giving 43.96 g of light orange crystals, mp 139–143°. [x] After heating the reaction mixture was boiled with methanol and cooled yielding 55.5% of yellow-tan crystals, mp 179–181°. [y] The reaction mixture was well mixed with pentane and dilute HCl and filtered. The solid was washed with water and dried giving 13.6 g of yellow-brown solid, mp about 249°. [z] The reactants were dissolved in ethanol which was evaporated and the residue heated as indicated. [aa] The dark red reaction mixture was allowed to stand at room temperature overnight and evaporated *in vacuo* below 50° to a dark oil which soon solidified. This was heated to boiling with methanol, cooled, and filtered, giving 11.6 g of yellow brown solid, mp 236–239.5° dec.

of radioactive lipids extracted from the carcass of mice that had been previously injected with ^{14}C-glucose (uniformly labeled).

Experimental Section[11]

General Procedure for Preparation of 2H-1,4-Benzoxazin-2-ones.—The requisite *o*-aminophenol and α-keto ester were mixed without solvent, under nitrogen, in the amounts given in Table I. The mixture was allowed to stand at room temperature or heated as indicated. The methods of working up the reaction mixture varied widely but unless otherwise indicated the product was heated to boiling with ethanol and cooled. The resulting solid was collected and recrystallized from the indicated solvent.

3-Methyl-2H-1,4-benzoxazin-2-one[2] (1).—A mixture of 10.9 g (0.1 mole) of *o*-aminophenol and 20.4 g (0.2 mole) of methyl pyruvate was allowed to stand at room temperature. The solid slowly dissolved giving a dark solution and in about 1 hr crystals started to separate. After standing several days the mixture was boiled with 100 ml of methanol dissolving most of the solid. After cooling, the mixture was filtered, giving 0.52 g of solid which was recrystallized from a mixture of DMF, methanol, and water; mp 245–247°. Its structure is unknown.

The methanol filtrate was evaporated and the residue recrystallized from 95% ethanol giving 11.34 g (70.5%) of brown crystals, mp 95–96°. A sample was purified by sublimation and recrystallization from ethanol; mp 101–103°.

A run under identical conditions using 23.6 g (0.2 mole) of ethyl pyruvate instead of methyl pyruvate became quite warm on standing. The resulting liquid did not crystallize even on seeding. On long standing it crystallized poorly. The solid was collected, washed with cold ethanol, and recrystallized from ethanol giving 2.7 g of cream-colored solid of unknown structure, mp 217–220°. Other runs at 100–130° using ethyl pyruvate gave up to 17% yields of 3-methyl-2H-1,4-benzoxazin-2-one.

4-Aminopyrogallol.[12]—A solution of 10.27 g (0.06 mole) of 4-nitropyrogallol[12] in 100 ml of 95% ethanol was hydrogenated with 0.2 g of platinum oxide catalyst at 3.5 kg/cm^2 and room temperature. After about 4 hr approximately the theoretical amount of hydrogen had been absorbed. A light red-brown solution containing considerable solid was obtained. This darkened rapidly in air and was kept under nitrogen as much as possible. The solid was dissolved by warming with more ethanol. The solution of the free base was filtered from the catalyst and used without isolation.

Hydrochloride.—When 1 equiv of HCl was added to the solution before hydrogenation, the uptake of hydrogen was much faster and the resulting solution of the hydrochloride was much lighter in color. However, even the hydrochloride tended to darken in air.

Ethyl 7-Methoxy-2-oxo-2H-1,4-benzoxazin-3-acetate (9).—A suspension of 15.3 g (0.1 mole) of 5-methoxy-2-nitrosophenol in 150 ml of ethanol and 8.26 ml (0.1 mole) of concentrated HCl was hydrogenated with 0.2 g of platinum oxide at room temperature and 3.5 kg/cm^2. Approximately the theoretical amount of hydrogen was absorbed in 4 hr and the uptake stopped. The brown solution was filtered from catalyst and 25.2 g (0.012 mole) of the sodium salt of diethyl oxalylacetate was added. After shaking for 0.5 hr the solvent was distilled and the dark residue was heated under nitrogen in a bath at 140–150° for 1 hr. The residue was dissolved in ethanol, filtered, and evaporated to dryness *in vacuo*. The residue was sublimed up to 200° (0.01 mm) giving a gummy solid. By fractional crystallization from ethanol a small sample of the desired compound was obtained.

7-Methoxy-3-phenyl-2H-1,4-benzoxazin-2-one (21).—A suspension of 20.6 g (0.134 mole) of 5-methoxy-2-nitrosophenol in 150 ml of ethanol was hydrogenated with 0.2 g of platinum oxide at room temperature and 3.5 kg/cm^2. About 83% of the theoretical amount of hydrogen was rapidly absorbed and the uptake stopped. The mixture contained crystalline solid which rapidly turned dark in air. To this mixture 17.8 g (0.1 mole) of ethyl phenylglyoxylate was added and the mixture was

(11) Melting points were taken in capillary tubes with a partial-immersion thermometer. Calibration of the apparatus against standard compounds showed no need for correction. Infrared and ultraviolet spectra were obtained on these compounds and were in accordance with the proposed structures.

(12) A. Einhorn, J. Cobliner, and H. Pfeiffer, *Ber.*, **37**, 100 (1904).

shaken under nitrogen for 1.5 hr. After removing the solvent and heating under nitrogen on a steam bath for 1 hr, the very dark residue was sublimed up to 188° (0.01 mm) giving about 5 g of crude solid. The sublimate was recrystallized from ethanol with the aid of Darco G-60, yielding 3.75 g of golden brown, silky needles mp 130–131.5°.

Ammonium Salt of 2-Oxo-3-phenyl-2H-1,4-benzoxazine-6-sulfonic Acid (22).—A solution of 14 g (0.05 mole) of technical (67.5%) *o*-aminophenol-*p*-sulfonic acid, 17.8 g (0.1 mole) of ethyl phenylglyoxylate, and 4 ml (0.06 mole) of aqueous NH_4OH in 25 ml of ethanol and 10 ml of water was boiled to dryness in an oil bath at 150° during 2 hr. The residue was boiled with 500 ml of ethanol, cooled, and filtered. The solid (7.3 g), mp 277–303°, appeared to be a mixture. The filtrate was concentrated and cooled giving 9 g (56%) of tan crystals, mp 292.5–294.5° dec. A sample recrystallized from ethanol with Darco G-60 treatment had the same melting point.

Methyl (4-Pyridyl)glyoxylate and Hydrate.—A mixture of 10.15 g (0.067 mole) of methyl 4-pyridylacetate, 10 ml of AcOH, 30 ml of benzene, and 7.44 (0.067 mole) of SeO_2 was stirred under reflux using a Dean–Stark trap. Approximately the theoretical amount of water was condensed in about 0.5 hr. The solvent was then removed *in vacuo* below 50° and the residue was distilled in a short-path apparatus. The product distilled below 150° (3 mm) giving 5.1 g of methyl 4-pyridylglyoxylate as a light yellow solid mixed with a little acetic acid. This was recrystallized from water from which the hydrate [methyl α,α-dihydroxy-α-(4-pyridyl)acetate] crystallized as light pink crystals, mp 114–118°, yield 2.33 g (19.0%). The structure of the hydrate was confirmed by infrared, ultraviolet, and nmr spectra and analysis.

Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65; O, 34.94; H_2O, 9.84. Found: C, 52.50; H, 4.79; N, 7.92; O, 34.70; H_2O, 9.86 (Karl Fischer).

3-(4-Pyridyl)-2H-1,4-benzoxazin-2-one (29).—A solution of 4.66 g (0.0254 mole) of methyl 4-pyridylglyoxylate hydrate and 2.79 g (0.0254 mole) of *o*-aminophenol in 50 ml of methanol was heated at 40° for 5 hr and evaporated to dryness *in vacuo* below 50°, and the residual oil was heated on a steam bath for 20 min. The resulting solid was shaken with 2% aqueous NaOH to dissolve phenolic material (see below). The product was collected, washed with water, dried, and sublimed up to 162° (0.001 mm) giving 2.6 g of solid. This was recrystallized from ethanol with the aid of Darco G-60 yielding 2.13 g of silky needles, mp 168–169°.

2'-Hydroxy-4-pyridineglyoxylanilide.—The above aqueous solution was acidified with acetic acid (pH 6) giving a solid which was collected, washed with water, and dried; 1.2 g, mp 212–213° dec. This was recrystallized from ethanol with Darco G-60 treatment yielding 0.7 g (11.4%) of the phenolic amide, mp 221.5–222.5° dec. The structure was confirmed by infrared, ultraviolet, and nmr spectra.

Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 64.46; H, 4.16; N, 11.57; O, 19.82. Found: C, 64.62; H, 4.08; N, 11.61; O, 20.39.

2-Carbethoxy-2-phenylbenzothiazoline.—A mixture of 35.7 g (0.2 mole) of ethyl phenylglyoxylate and 25.1 g (0.2 mole) of *o*-aminobenzenethiol was heated under nitrogen in an oil bath at 135–150° for 1 hr. After cooling the orange-yellow liquid crystallized. This was well mixed with pentane and dilute HCl and filtered. The solid was washed with water and dried giving 30.8 g (54%) of waxy crystals, mp 77–97°. This was recrystallized from 150 ml of 95% ethanol yielding 20.1 g of light yellow crystals, mp 97.5–99.5°. Infrared and ultraviolet spectra and analysis are in agreement with the benzothiazoline structure.

Anal. Calcd for $C_{16}H_{15}NO_2S$: C, 67.34; H, 5.30; N, 4.91; S, 11.24. Found: C, 67.37; H, 5.37; N, 4.79; S, 11.67.

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2-Amino-5-Substituted 1,3,4-Oxadiazoles and 5-Imino-2-Substituted Δ^2-1,3,4-Oxadiazolines. A Group of Novel Muscle Relaxants

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The synthesis of 2-amino-5-substituted 1,3,4-oxadiazoles by the reaction of a 1-acyl-3-thiosemicarbazide with Pb_3O_4 is described. One of the procedures, namely the use of Pb_3O_4 in N,N-dimethylformamide, has some advantages over the earlier literature methods. Several of the oxadiazoles are highly potent in producing a profound flaccid paralysis in laboratory animals. Structure–activity relationships in this series of compounds are discussed. Protonation of these 2-amino-1,3,4-oxadiazoles produces large hypsochromic shifts of the endocyclic >C=N- band seen in the infrared spectra of the bases, thus indicating preferential formation of an endocyclic cation. This phenomenon is not observed with the 2-amino-1,3,4-thiadiazoles, 2-aminooxazoles, 2-aminooxazolines, and 2-aminothiazoles; in these systems, protonation occurs at the exocyclic NH_2.

During the pharmacological evaluation of a number of heterocycles, the observation was made that 2-acetamido-5-phenyl-1,3,4-oxadiazole (**28**) produced a profound flaccid paralysis in rats.[1] Subsequent investigation showed that this activity was shared by the parent base, 2-amino-5-phenyl-1,3,4-oxadiazole (**20**), and its hydrochloride (**22**)[2] and a number of other 2-amino-5-aryl-1,3,4-oxadiazoles. These compounds, their physical properties, and analytical data are shown in Table II. This paper will discuss their structure and synthesis and will present a structure–activity relationship in this and related heterocyclic systems.

Structure.—The 2-amino-1,3,4-oxadiazoles, when visualized as cyclic amidines, would be expected to show spectrophotometric differences upon protonation.[3] The infrared spectral data[4] now being reported show that protonation invariably produced a large hypsochromic

(1) This observation was first made by Dr. J. J. Piala of these laboratories.

(2) The detailed pharmacologic and toxicologic studies made with this compound were reported by G. L. Hassert, Jr., J. W. Poutsiaka, D. Papandrianos, J. C. Burke, and B. N. Craver, *Toxicol. Appl. Pharmacol.*, **3**, 726 (1961).

(3) This phenomenon has been discussed by B. Witkop, *Experientia*, **10**, 420 (1954), insofar as it is concerned with the aminopyridines, α-aminoindolenine, and a number of alkaloids.

(4) The insolubility of these compounds has necessitated determining the infrared spectra on mineral oil mulls. This lack of solubility has also led to inconclusive deuterium exchange studies and has made impossible nmr spectral studies except with a few of the compounds prepared.