A Chemical and Pharmacological Study of Some Compounds Derived from 3,4-Xylidine

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Twelve derivatives of 3,4-xylidine, consisting of three simple related xylidines, two quinolines, a cinnoline and six dimethylbenz[f]azepines, were synthesized as potential antimetabolites of riboflavin and vitamin B_{12}. They were examined for toxicity, pharmacological activity and screened chemotherapeutically against malaria, trypanosomiasis, yellow fever and Crocker sarcoma 180 in mice. Two of the dimethylbenz[f]azepine derivatives showed marked activity against Crocker sarcoma, the most active compound comparing favorably in activity with a therapeutically active antitumor drug, triethanomelamine. Two other closely related compounds bearing the same heterocyclic ring showed weaker antitumor activity but these were not well tolerated in the strain of mice used for tumor work. The compounds were, on the whole, well tolerated and no demonstrable pharmacological effects were elicited at relatively high concentrations. None of the compounds tested showed activity against experimental infections of malaria, trypanosomiasis or yellow fever.

Derivatives of 3,4-xylidine were subjected to biological testing with the hope that they might prove to be antimetabolites of riboflavin and vitamin B_{12}. Riboflavin has been shown to play some role in the development of malarial and trypanosomal infections,[1,2] and also in certain forms of tumor growth.[3] There is evidence that vitamin B_{12} is synthesized by spontaneous mammary tumors[4] on which the tissue is partly dependent,[5] and a simple xylidine derivative used as a potential antimetabolite caused temporary regression of the tumor.[6] Other related xylidine derivatives have been shown to be active against influenza virus,[7] and this led us to include a test for antiviral activity.

The compounds include three simple related xylidines, two quinolines, a cinnoline and six dimethyl benz[f]azepine derivatives.

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(2) R. G. Yaeger and O. N. Miller, *Exper. Parasitol.*, **10**, 227 (1960).
(3) H. C. Stoerk and G. A. Emerson, *Proc. Soc. Exp. Biol.* N. Y., **70**, 703 (1949).
(4) D. W. Woolley, *Proc. Nat. Acad. Sci. U. S. A.*, **39**, 6 (1953).
(5) D. W. Woolley, "Antimetabolites and Cancer," American Assoc. for Advancement of Science, Washington, D. C., 1955, p. 163.
(6) D. W. Woolley and G. Schaffner, *Cancer Res.*, **14**, 802 (1954).
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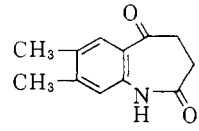
1. N-3,4-Dimethylphenylsuccinamic acid[8]
2. 2-β-Carboxypropionyl - 4,5 - dimethylacetanilide[8]
3. 2-β-Carboxypropionyl-4,5-dimethylaniline hydrochloride hydrate[8]
4. Sodium 2,6,7-trimethyl-4-hydroxyquinoline-3-acetate dihydrate
5. 6,7-Dimethyl-2-hydroxyquinoline - 4 - carboxylic acid acetate[8]
6. 6,7-Dimethyl- 4 - hydroxycinnoline - 3 - acetic acid
7. 2,3,4,5-Tetrahydro-7,8-dimethyl-2,5,-dioxobenz[f]azepine[8]
8. 4-(*p*-Dimethylaminophenylamino)-2,5,-dihydro-6,7-dimethyl-2,5-dioxobenz[f]-azepine[8]
9. 4-(Piperidinomethyl)-2,5-dihydro-7,8-dimethyl-2,5-dioxobenz[f]azepine
10. 4-(Methyl-1-piperazinomethyl)-2,5,-dihydro-7,8-dimethyl-2,5-dioxobenz[f]-azepine
11. 4-Hydroxy-2,5-dihydro-7,8-dimethyl-2,5-dioxobenz[f]azepine[8]
12. 3-(Piperidinomethyl)-4-hydroxy-2,5-dihydro-7,8-dimethyl-2,5-dioxobenz[f]-azepine



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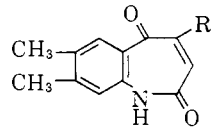
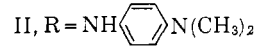
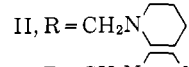


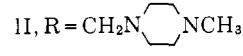
II



II, R = NH–C6H4–N(CH3)2



II, R = CH2N(C5H10)



II, R = CH2N(C4H8)NCH3

Experimental

2,4,6-Trimethyl-4-hydroxyquinoline-3-acetic Acid (Compound 4).—N-Acetyl-2-β-carboxypropionyl-4,5-dimethylaniline (13.2 g.) was heated ovenight on a water-bath with 2 *N* NaOH (55 ml.). Charcoal was added, and after filtering the solution was left to cool when a sodium salt (8.6 g.) separated, m.p. 360° dec. (sublim.) (from water).

Anal. Calcd. for $C_{14}H_{14}NNaO_3 \cdot 2H_2O$: C, 55.5; H, 6.0; N, 4.6; Na, 7.6. Found: C, 55.7; H, 6.1; N, 4.75; Na, 7.25.

The ultraviolet spectrum showed maxima at 220, 245 and 320.5 mμ with log ε 1.99, 2.15 and 1.38, respectively. Neutralization of a dilute solution of the salt gave the free acid, no m.p. below 420° (from dimethylformamide).

Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.5; H, 6.2; O, 19.6; N, 5.7; acetyl, none. Found: C, 68.6; H, 6.2; O, 20.0; N, 5.9; acetyl, nil.

This compound gave a red ferric chloride color and formed an unstable hydrochloride from strong acid solution. Because of these facts the 4-quinolone structure was preferred to the alternative 2-quinolone, also feasible.[9,10]

6,7-Dimethyl-4-hydroxycinnoline-3-acetic Acid (Compound 6).[11]—2-β-Carboxypropionyl-3,4-dimethylaniline (5.9 g.) was stirred with 2 *N* HCl (67 ml.) at 5°. Sodium nitrite solution (10%, 20 ml.) was added dropwise and the re-

(8) A. H. Rees, *J. Chem. Soc.*, 3111 (1959).
(9) R. Camps, *Ber.*, **32**, 3228 (1899).
(10) E. Wohnlich, *Arch. Pharm.*, **251**, 526 (1913).
(11) K. Schofield and J. C. E. Simpson, *J. Chem. Soc.*, 520 (1945).

sulting solution was warmed. The precipitate which appeared was collected (4.8 g.) and recrystallized from acetic acid, m.p. 310° dec.

Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.05; H, 5.2; N, 12.05; O, 20.6. Found: C, 62.4; H, 5.25; N, 11.5; O, 21.2.

4-N-Substituted Aminomethyl Derivatives of 2,5-Dihydro-7,8-dimethyl-2,5-dioxobenz[f]azepine.—To the dione (1 g.) in propanol was added the base (1 ml.) and 40% formaldehyde solution (0.8 ml.). The mixture was heated until the dione had dissolved, filtered and left to cool when the Mannich base separated. These bases were considered to be the 4-substituted derivatives as their infrared spectra retained the peaks at 3.1 μ (m) and 3.3 μ (w) present in the starting dione. By this method were obtained (i) the **Morpholinomethyl derivative** which originally was prepared to characterize the dione and has not been tested. It had m.p. 245° (from propanol).

Anal. Calcd. for $C_{17}H_{20}N_2O_3$: C, 67.5; H, 7.1; N, 8.7. Found: C, 67.9; H, 6.7; N, 9.3.

The base picrate had m.p. 200° (from propanol).

Anal. Calcd. for $C_{23}H_{23}N_5O_{10}$: N, 13.2. Found: N, 13.0.

(ii) **Piperidinomethyl derivative (Compound 9)**, m.p. 210° (from propanol).

Anal. Calcd. for $C_{18}H_{22}N_2O_2$: C, 72.45; H, 7.4; N, 9.4. Found: C, 72.7; H, 7.6; N, 9.4.

The *picrate* had m.p. 230° (from dioxane).

Anal. Calcd. for $C_{24}H_{25}N_5O_{10}$: N, 13.3. Found: N, 13.35.

(iii) **N′-Methylpiperazinomethyl derivative (Compound 10)**, m.p. 208° (from propanol).

Anal. Calcd. for $C_{18}H_{23}N_3O_2$: C, 69.0; H, 7.4; N, 13.4. Found: C, 69.0; H, 7.2; N, 13.0.

The *dipicrate* had m.p. 252° (from dioxane).

Anal. Calcd. for $C_{30}H_{29}N_9O_{16}$: N, 16.35. Found: N, 16.2.

2,5-Dihydro-4-hydroxy-7,8-dimethyl-2,5-dioxobenz[f]azepine (11) was prepared according to the literature[8] and purified by recrystallization from acetic acid or dimethylformamide, m.p. 295°. It was characterized as the quinoxaline derivative, m.p. 352° (from acetic acid).

Anal. Calcd. for $C_{18}H_{13}N_3O$: C, 74.7; H, 5.2; N, 14.5. Found: C, 74.8; H, 5.3; N, 14.3.

3-(Piperidinomethyl)-4-hydroxy-2,5-dihydro-7,8-dimethyl-2,5-dioxobenz[f]-azepine (Compound 12) was obtained by the general method already described. It was not soluble in propanol, nor could it be recrystallized from dioxane, pyridine or dimethylformamide as it was not stable to heat in these solvents, nonbasic material being obtained. It was purified by washing with alcohol, m.p. 220°.

Anal. Calcd. for $C_{18}H_{22}N_2O_3$: C, 68.8; H, 7.0; N, 8.9. Found: C, 68.8; H, 7.0; N, 8.65.

The *picrate* had m.p. 230°.

Anal. Calcd. for $C_{24}H_{23}N_5O_{10}$: N, 12.9. Found: N, 12.15.

Methods and Results

Solubility.—Compounds 1, 2, 4, 5 and 6 were soluble in saline or saline containing 5% $NaHCO_3$, and which could be neutralized without precipitation. Compound 3 gave a very acid solution in saline which precipitated readily on neutralization. Compounds 7 to 12 were insoluble and were used as suspensions.

All except compounds 8 and 11 were soluble in propylene glycol and solutions in this solvent were used for isolated tissue preparations.

Toxicity and Dosage.—LD_{50} values were determined in mice using the intraperitoneal route of administration. The maximum tolerated dose (MTD) intraperitoneally in mice given daily for 8 days was determined in order to establish a dose for the chemotherapeutic tests. The drugs were administered at the MTD in solution or suspension daily by intraperitoneal injection for all *in vivo* tests.

Table I

Compound	1	2	3	4	5	6
LD_{50}, mg./kg.	1000	500	250	2000	1500	500
MTD, mg./kg.	125	250	50	250	250	250
Compound	7	8	9	10	11	12
LD_{50}, mg./kg.	>2000	>2000	300	50	>2000	>2000
MTD, mg./kg.	250	250	12.5	5	25	50

Antimalarial Test.—Mice infected[12] with a strain of *Plasmodium berghei* were treated with the compounds and their activity in reducing parasitaemia was determined and compared with that of known antimalarials. None of the compounds was active.

Antitrypanosomal Test.—The compounds were examined for an effect on the survival time of mice infected with *Trypanosoma brucei*.[13]

Antitumor Activity.—Crocker sarcoma 180 was employed using a modification of a method previously described.[14] Groups of 10 mice were used and treatment was given once daily for 10 days. The tumors were weighed 14 days after implantation and the weight expressed in g. per 20 g. mouse. The results of the treated groups were expressed relative to untreated controls and compared with the results obtained with triethanomelamine (TEM) at the MTD in mice.

Two of the compounds (7 and 8) showed marked antitumor activity com-

Table II

Effect of Dimethylbenz[f]azepine Derivatives on Crocker Sarcoma 180

Drug	Daily dose mg./20 g. mouse	Average tumor weight g./20 g. mouse	Survivors	% of control tumor	P
Nil	—	1.59 ± 0.20[b]	9/10	100	—
7	5.0	0.42 ± 0.18	8/10	26	0.02
8	5.0	0.65 ± 0.32	8/10	41	0.05
9	0.25	1.05 ± 0.55	3/10	66	N.S.[c]
10	0.10[a]	1.34 ± 0.25	4/10	84	N.S.
T.E.M.	0.015	0.34 ± 0.28	4/5	21	0.02

[a] Given on alternate days. [b] Standard error. [c] Not significant.

(12) J. P. Thurston, *Brit. J. Pharmacol.*, **5**, 409 (1950).

(13) J. H. Burn, D. J. Finney, and L. G. Goodwin, "Biological Standardization," 2nd ed., Oxford University Press, London, p. 379.

(14) C. C. Stock, "Advances in Cancer Research," Vol. II, Academic Press, New York, N. Y., p. 433.

parable to that produced by TEM. There was also some indication of activity amongst compounds 9 and 10 but these were poorly tolerated in the mice used for the tumor work.

Antiviral Test.—All except compounds 6, 10 and 12 were examined for activity against neurotropic yellow fever virus developing in mice.[15] None of the compounds tested was active.

Pharmacological Tests.—The compounds were tested for activity against isolated guinea-pig ileum, rabbit auricles, rat blood pressure and for any action they might have in modifying the effect of histamine, acetylcholine, epinephrine and 5-hydroxytryptamine on these tissues. The response of rat phrenic nerve-diaphragm preparation to nerve and muscle stimulation was also investigated.

Concentrations of up to 1 mg./ml. of all the compounds were ineffective on isolated tissue preparations, and doses of 40 mg./kg. were ineffective in rat blood pressure experiments.

Discussion

The only activity established in this group of compounds is that against experimental cancer, and this activity is found exclusively among the dimethylbenz[f]azepine derivatives. Compound 7 (I) was the most active and was obtained by ring closure of a simple xylidine derivative (compound 3) which itself was devoid of antitumor activity. One would therefore conclude that this heterocyclic ring is essential for activity. The compounds which exert activity against sarcoma 180 comparable with that of a known therapeutic agent clearly should be subjected to a wide tumor spectrum and other related derivatives tested. This work is already in progress.

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(15) F. Macnamara, personal communication.