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Research Papers

Some cyclic hydroxamic acids

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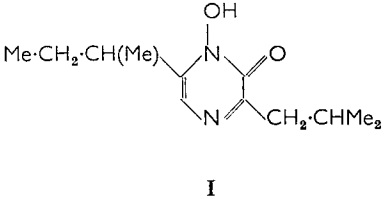
The preparation of certain quinolines, quinazolines, quinoxalines, benzoxazines and benzothiazines containing the cyclic hydroxamic grouping is described. *In vitro* antibacterial activities showed that no compound had a broader spectrum of activity than the known 1,2-dihydro-1-hydroxy-2-oxoquinoline.

THE discovery of the high inhibitory *in vitro* activity of aspergillic acid (I) against both Gram-positive and Gram-negative organisms (White, 1940; White & Hill, 1943), together with the demonstration of a hydroxamic acid grouping in the molecule (Dutcher, 1947; Dunn, Gallagher, Newbold & Spring, 1948), stimulated a search for less toxic analogues. Despite the fact that over the last 20 years at least twelve different aromatic *N*-heterocyclic systems containing a hydroxamic acid grouping have been synthesised, the preparative difficulties have, in most instances, precluded the synthesis of more than a few examples of each particular system. The preparation of compounds closely related to the naturally occurring cyclic hydroxamic acids aspergillic acid (I), pulcherriminic acid (II) and iodinin (III) remains a moderately difficult task. The synthesis of bi- and tri-cyclic systems containing a hydroxamic group has, however, been facilitated by the discovery (Coutts & Wibberley, 1963) that reductive cyclisation of suitable *o*-nitro-esters with sodium borohydride and palladised charcoal yielded cyclic hydroxamic acids.

In view of the known superior antibacterial activity of 1,2-dihydro-1-hydroxy-2-oxoquinoline (IV; X–Y = CH = CH) (see Newbold & Spring, 1948) we have prepared a number of analogues of this compound which retain a bicyclic ring system but have other heteroatoms within the molecule. The influence of aromaticity on antibacterial activity has also been investigated both in the parent quinolines and in aza-analogues.



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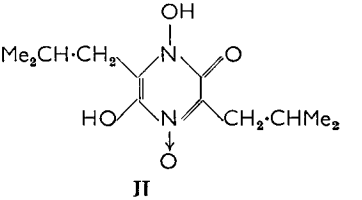

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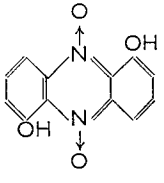

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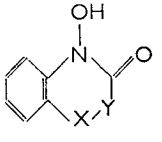

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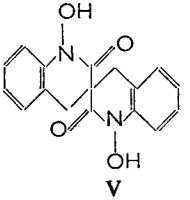
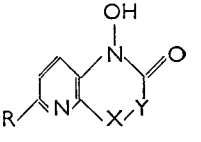


V



VI

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Ethyl β-(*o*-nitrophenyl)propionate gave a good yield of 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline (IV; X–Y = CH_2—CH_2) on treatment with sodium borohydride and palladised charcoal, and the precursor of this ester, ethyl *o*-nitrobenzylmalonate gave the related 3-ethoxycarbonyl-1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline. The reduction of ethyl di(*o*-nitrobenzyl)malonate, a by-product in the synthesis of the *o*-nitrobenzylmalonate yielded the first spiro-cyclic hydroxamic acid, 3,3′-spirobi-(1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline) (V) to be reported.

We have previously described (Coutts & Wibberley, 1963) the preparation of the benzothiazines (IV; X–Y = S–CH_2 and SO_2–CH_2), the antibacterial properties of which are recorded in Table 1. The closely related 3,4-dihydro-4-hydroxy-3-oxo-2*H*-1,4-benzoxazine (IV; X–Y = O–CH_2) was obtained from ethyl *o*-nitrophenoxyacetate in a higher yield than that found by Honkanen & Virtanen (1960), who reduced the ester with zinc and ammonium chloride. The isomeric 1,2-dihydro-1-hydroxy-2-oxo-4*H*-3,l-benzoxazine (IV; X–Y = CH_2–O) was similarly prepared from methyl *o*-nitrobenzyl carbonate.

The synthesis of 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinazoline (IV; X–Y = CH_2–NH) was accomplished from either methyl *o*-nitrobenzylcarbamate or from diethyl *o*-nitrobenzylidenebiscarbamate in only moderate yield, as also was the synthesis of 1,2-dihydro-1,4-dihydroxy-2-oxoquinazoline by the reduction of ethyl *o*-nitrobenzoylcarbamate. Oxidation of the former tetrahydroquinazoline to the fully aromatic 1,2-dihydro-1-hydroxy-2-oxoquinazoline (IV; X–Y = CH=N) was accomplished by passing a solution of the compound through an ion-exchange resin containing ferric ions. The isomeric 3,4-dihydro-3-hydroxy-4-oxoquinazoline was prepared by the method of Adachi (1957).

Ethyl *N*-*o*-nitrophenylglycine was best prepared by the interaction of glycine and 1,2-dinitrobenzene (cf Crowther, Curd, Davey & Stacey, 1949, for the synthesis of the 5-chloro-analogue). On reduction it gave the expected 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoxaline (IV; X–Y = NH–CH_2), but when the nitrogen atmosphere maintained during reduction was removed and the reaction mixture stirred for several hours, atmospheric oxidation occurred to yield the corresponding 1,2-dihydroquinoxaline (IV; X–Y = N=CH). The latter was identical with the product obtained by the action of acetic anhydride on quinoxaline 1,4-dioxide (cf Elina, 1962). 1,2-Dihydro-1,3-dihydroxy-2-oxoquinoxaline was only obtained in very low yield by the reductive cyclisation of *N*-ethoxalyl-*o*-nitroaniline and was therefore prepared by the method of Tennant (1963).

Two examples of triazanaphthalenes containing the cyclic hydroxamic acid grouping were prepared. Reduction of 2-ethoxycarbonylmethylamino-3-nitropyridine yielded 1,2,3,4-tetrahydro-1-hydroxy-2-oxo-1,4,5-triazanaphthalene (VI; R=H, X–Y = NH–CH_2), and reduction of 2-ethoxalylamino-6-methyl-3-nitropyridine yielded 1,2-dihydro-1,3-dihydroxy-6-methyl-2-oxo-1,4,5-triazanaphthalene (VI; R=Me, X–Y = N=C(OH)).

All the cyclic hydroxamic acids gave wine-red or blue colours with aqueous ferric chloride solution and were soluble in sodium hydrogen

carbonate solution. Their infra-red spectra showed carbonyl and hydroxyl absorption.

ANTIBACTERIAL ACTIVITIES

The results of antibacterial screening tests are in Table 1. Previous reported figures for minimal inhibitory concentration of 1,2-dihydro-1-hydroxy-2-oxoquinoline (Newbold & Spring, 1948) were 2·0 and 1·0 mg/100 ml against *Staphylococcus aureus* and *Escherichia coli* respectively. None of the new compounds has an activity surpassing this although several compounds show more specific activity against *Staph. aureus*.

TABLE 1. MIC (MG/100 ML) OF CYCLIC HYDROXAMIC ACIDS

Compound	Test organisms				
	Staph. aureus	*B. subtilis*	*E. coli*	*S. typhi*	*P. vulgaris*
1,2,3,4-Tetrahydro-1-hydroxy-2-oxoquinoline	40	20	40	>40	>40
3-Ethoxycarbonyl-1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline	40	>40	>40	>40	>40
3,3′-Spirobi-(1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline)*	>40	>40	>40	>40	>40
3,4-Dihydro-4-hydroxy-3-oxo-1,4-benzothiazine[1]	>40	>40	>40	>40	>40
3,4-Dihydro-4-hydroxy-3-oxo-1,4-benzothiazine 1,1-dioxide[1]	>40	>40	>40	>40	>40
3,4-Dihydro-4-hydroxy-3-oxo-2*H*-1,4-benzoxazine	10	40	>40	>40	>40
1,2-Dihydro-1-hydroxy-2-oxo-4*H*-3,1-benzoxazine	40	>40	>40	>40	>40
1,2,3,4-Tetrahydro-1-hydroxy-2-oxoquinazoline	5	40	>40	>40	>40
1,2-Dihydro-1,4-dihydroxy-2-oxoquinazoline	>40	>40	>40	>40	>40
1,2-Dihydro-1-hydroxy-2-oxoquinazoline	20	40	>40	>40	>40
3,4-Dihydro-3-hydroxy-4-oxoquinazoline[2]	>40	>40	>40	>40	>40
1,2,3,4-Tetrahydro-1-hydroxy-2-oxoquinoxaline	20	>40	>40	>40	>40
1,2-Dihydro-1-hydroxy-2-oxoquinoxaline	20	>40	>40	>40	>40
1,2-Dihydro-1,3-dihydroxy-2-oxoquinoxaline[3]	>40	>40	>40	>40	>40
1,2,3,4-Tetrahydro-1-hydroxy-2-oxo-1,4,5-triazanaphthalene	5	40	>40	>40	>40
1,2-Dihydro-1,3-dihydroxy-methyl-2-oxo-1,4,5-triazanaphthalene	40	40	>40	>40	>40
1,2-Dihydro-1-hydroxy-2-oxoquinoline[4]	10	5	5	5	10

[1] Coutts and Wibberley, 1963.
[2] Adachi, 1957.
[3] Tennant, 1963.
[4] Ohta and Ochiai, 1962.
* Tested as a suspension in ethanol.

Experimental

General method of reductive cyclisation. A solution of the nitro-ester (0·1 mole) in dioxan was added over 5 min to a suspension of palladised charcoal (0·2 g) in 2% sodium hydroxide solution (20 ml) containing sodium borohydride (0·025 mole). A stream of nitrogen was passed through the stirred mixture for the stated time, the catalyst was removed by filtration, and the product isolated by suitable means from the acidified filtrate.

1,2,3,4-*Tetrahydro*-1-*hydroxy*-2-*oxoquinoline* (IV; X–Y = CH_2–CH_2). Reduction of ethyl β-(*o*-nitrophenyl)propionate (15 min) yielded, by extraction with chloroform, the *quinoline* (75%) as colourless plates (from ethanol), m.p. 117–118°. Found: C, 66·2; H, 5·7; N, 8·8. $C_9H_9NO_2$ requires C, 66·25; H, 5·5; N, 8·6%. ν_{max} 2,700–3,200 w (O–H), 1,690 s cm^{-1} (C=O).

3-*Ethoxycarbonyl*-1,2,3,4-*tetrahydro*-1-*hydroxy*-2-*oxoquinoline*. Reduction of ethyl *o*-nitrobenzylmalonate (30 min) yielded, by extraction with ether, the *ethoxycarbonylquinoline* (39%) as colourless needles (from ethanol), m.p. 137–139°. Found: C, 61·7; H, 5·8; N, 6·0. $C_{12}H_{13}NO_4$ requires C, 61·3; H, 5·5; N, 6·0%. ν_{max} 3,000–3,200 w (O–H) 1,715 s (ester C=O) 1,665 s cm^{-1} (ring C=O).

3,3′-*Spirobi*-(1,2,3,4-*tetrahydro*-1-*hydroxy*-2-*oxoquinoline*) (V). Reduction of ethyl di(*o*-nitrobenzyl)malonate (15 min) yielded, by filtration, the *spiro hydroxamic acid* (V) (76%). The solubility of this compound in organic solvents was extremely low, and purification was effected by repeated dissolution in alkali and precipitation with acid to constant m.p., followed by washing with hot acetic acid. Found: C, 65·45; H, 4·8; N, 8·7. $C_{17}H_{14}N_2O_4$ requires C, 65·8; H, 4·5; N, 9·0%. ν_{max}3,050–3,250 m (O–H), 1,650 s, 1,670 sh cm^{-1} (C=O).

3,4-*Dihydro*-4-*hydroxy*-3-*oxo*-2H-1,4-*benzoxazine* (IV; X–Y = O–CH_2). Reduction of ethyl *o*-nitrophenoxyacetate (15 min) yielded, on filtration, the *benzoxazine* (84%) as colourless needles (from aqueous ethanol), m.p. 156° with prior sublimation. Found: C, 58·5; H, 4·2; N, 8·5. Calc for $C_8H_7NO_3$: C, 58·2; H, 4·2; N, 8·5%. ν_{max} 2,650–3,250 m (O–H) 1,650 s, 1,680 s cm^{-1} split (C=O), Honkanen & Virtanen (1960) obtained the same product in 60% yield in a reduction with zinc and ammonium chloride.

Methyl o-*nitrobenzyl carbonate*. A solution of *o*-nitrobenzyl alcohol (7·3 g), methyl chloroformate (7·3 ml) and pyridine (3·8 ml) in chloroform (40 ml) was stirred at room temperature for 14 hr. The solution was washed with water, dried (Na_2SO_4) and distilled to yield the *carbonate* (64%), b.p. 132–134°/0·9 mm as colourless prisms (from ethanol), m.p. 46–47°. Found: C, 51·3; H, 4·35; N, 7·1. $C_9H_9NO_5$ requires C, 51·2; H, 4·3; N, 6·6%.

1,2-*Dihydro*-1-*hydroxy*-2-*oxo*-4*H*-3,1-*benzoxazine* (IV; X–Y = CH_2–O). Reduction of methyl *o*-nitrobenzyl carbonate (25 min) yielded, by extraction with chloroform, the *benzoxazine* (33%) as colourless prisms (from benzene), m.p. 127–128°. Found: C, 57·8; H, 4·2; N, 8·0. $C_8H_7NO_3$ requires C, 58·2; H, 4·2; N, 8·5%. ν_{max} 3,000–3,200 m (O–H), 1,690 s cm^{-1} (C=O).

Diethyl o-*nitrobenzylidenebiscarbamate*. *o*-Nitrobenzaldehyde (3·0 g) and ethyl carbamate (3·6 g) were melted together on a water-bath. Concentrated hydrochloric acid (0·2 ml) was added and the mixture heated for 30 min. The residue was washed with water and ethanol to leave the *carbamate* (5·1 g) as colourless needles (from 2-ethoxyethanol), m.p. 179–181°. Found: C, 50·6; H, 5·6; N, 13·7. $C_{13}H_{17}N_3O_6$ requires C, 50·2; H, 5·5; N, 13·5%.

Methyl o-*nitrobenzylcarbamate*. *o*-Nitrobenzylamine (0·7 g), methyl chloroformate (0·47 g), potassium carbonate (0·7 g) and ether (30 ml) were refluxed for 7 hr. Evaporation of the ethereal layer, after filtering off the inorganic material, gave the *carbamate* (0·76 g) as colourless needles (from ethanol), m.p. 84–85°. Found: C, 51·5; H, 4·75; N, 13·3. $C_9H_{10}N_2O_4$ requires C, 51·4; H, 4·8; N, 13·3%.

1,2,3,4-*Tetrahydro*-1-*hydroxy*-2-*oxoquinazoline* (IV; X–Y = CH_2–NH). Reduction of diethyl *o*-nitrobenzylidenebiscarbamate (2 hr) yielded, on concentration to low volume, the *quinazoline* (30%), m.p. 167–169° (from ethanol). Found: C, 58·7; H, 4·7; N, 16·3. $C_8H_8N_2O_2$ requires C, 58·5; H, 4·9; N, 17·1%. ν_{max} 3,350 w (free N–H), 3,050–3,300 w (bonded) O–H and/or NH), 1,665 s cm^{-1} (C=O). Reduction of methyl *o*-nitrobenzylcarbamate in the absence of dioxan, followed by extraction with ether, yielded the same quinazoline (m.p. and mixed m.p. 167–169°) (39%).

Ethyl o-nitrobenzoylcarbamate. *o*-Nitrobenzoyl chloride (70 g) was added over 15 min to ethyl carbamate (140 g) at 160° (oil-bath). The mixture was heated for a further 15 min at 160°, cooled, dissolved in ether, and the solution extracted with 5N sodium hydroxide. Acidification of the extract yielded the *carbamate* (15 g) as colourless needles (from aqueous acetic acid), m.p. 128–129°. Found: C, 50·55; H, 4·3; N, 11·9. $C_{10}H_{10}N_2O_5$ requires C, 50·4; H, 4·2; N, 11·8%.

1,2-*Dihydro*-1,4-*dihydroxy*-2-*oxoquinazoline.* Reduction of ethyl *o*-nitrobenzoylcarbamate in 10% sodium hydroxide solution (15 min) yielded, by filtration, the *quinazoline* (33%) as colourless needles (from acetic acid), m.p. 282° (decomp.). Found: C, 53·4; H, 3·2; N, 16·15. $C_8H_6N_2O_3$ requires C, 53·9; H, 3·4; N, 15·7%. ν_{max} 3,000–3,150 w (O–H), 1,690 s cm^{-1} (C=O).

1,2-*Dihydro*-1-*hydroxy*-2-*oxoquinazoline* (IV; X–Y = CH=N). Aqueous ferric chloride solution was passed through a strongly acidic cation-exchange resin until no more ferric ion was adsorbed. The column was washed thoroughly with water, and a solution of 1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinazoline (0·8 g) in 2% sodium hydroxide solution (20 ml) passed through. The eluate was neutralised, evaporated to dryness and extracted into absolute ethanol. Evaporation of the extract yielded the *quinazoline* (0·15 g) as cream needles (from water), m.p. 212–213°. Found: C, 59·05; H, 3; N, 17·4. $C_8H_6N_2O_2$ requires C, 59·3; H, 3·7; N, 17·3%. ν_{max} 2,350–2,700 w (O–H), 1,665 s cm^{-1} (C=O).

1,2,3,4-*Tetrahydro*-1-*hydroxy*-2-*oxoquinoxaline* (IV; X–Y = NH–CH_2). Reduction of ethyl *N*-*o*-nitrophenylglycine (prepared in 80% yield from 1,2-dinitrobenzene by the method of Crowther, Curd, Davey & Stacey, 1949, for the 5-chloro analogue) in 5% sodium hydroxide solution yielded, by extraction with ether, the *quinoxaline* (66%) as colourless prisms (from ethanol), m.p. 156° (decomp.). Found: C, 58·35; H, 5·0; N, 17·0. $C_8H_8N_2O_2$ requires C, 58·5; H, 4·9; N, 17·1%. ν_{max} 3,200 m (N–H) 2,350–2,700 w (O–H), 1,685 s cm^{-1} (C=O).

1,2-*Dihydro*-1-*hydroxy*-2-*oxoquinoxaline* (IV; X–Y = N=CH). When the preceding reduction of ethyl *N*-*o*-nitrophenylglycine was carried out in the absence of nitrogen and the reaction mixture was stirred vigorously for a further 6 hr, atmospheric oxidation took place to yield, on acidification, the 1,2-*dihydroquinoxaline* (49%). Sublimation followed by crystallisation from ethanol gave cream needles, m.p. 210–211°. Found: C, 59·45; H, 3·9; N, 17·3. Calc for $C_8H_6N_2O_3$: C, 59·3; H, 3·7; N, 17·3%. ν_{max} 2,350–2,700 w (O–H), 1,645 s cm^{-1} (C=O). The action of acetic

anhydride on quinoxaline 1,4-dioxide gave the same hydroxamic acid, m.p., and mixed m.p. 210–211° in 18% yield. (Elina, 1962, states 23% yield, m.p. 208–209°).

1,2-*Dihydro*-1,3-*dihydroxy*-2-*oxoquinoxaline*. Reduction of *N*-ethoxalyl-*o*-nitroaniline (prepared by method of Reindel & Rosendahl, 1962, for 2-ethoxalylaminopyridine) (10 min) yielded the *quinoxaline* (15%), m.p. and mixed m.p. 292° (decomp.) with a sample prepared from 3-hydroxyquinoxaline 1-oxide by the method of Tennant, 1963. ν_{max} 2,500–3,200 w (O–H), 1,670 and 1,705 s cm^{-1} (C=O).

1,2,3,4-*Tetrahydro*-1-*hydroxy*-2-*oxo*-1,4,5-*triazanaphthalene* (VI; R=H, X–Y = NH–CH_2). Reduction of 2-ethoxycarbonylmethylamino-3-nitropyridine (Albert & Barlin, 1963) in 2% sodium hydroxide solution yielded directly the *triazanaphthalene* (46%) as colourless needles (from ethanol), m.p. 192° (decomp.). Found: C, 51·05; H, 4·45; N, 25·1. $C_7H_7N_3O_2$ requires C, 50·9; H, 4·2; N, 25·45%. ν_{max} 3,300 m (N–H), 2,300–2,700 w (O–H), 1,665 s cm^{-1} (C=O).

2-*Ethoxalylamino*-6-*methyl*-3-*nitropyridine*. 2-Amino-3-nitro-6-methylpyridine (11·25 g), pyridine (25 ml) and ethoxalyl chloride (10 g) were stirred together for 15 min. The mixture was poured into dilute hydrochloric acid and extracted with ether. The extract was evaporated to yield the *ester* (9·7 g) as orange needles (from ethanol), m.p. 106–107°. Found: C, 47·4; H, 4·3; N, 17·5. $C_{10}H_{11}N_3O_5$ requires C, 47·4; H, 4·35; N, 16·6%.

1,2-*Dihydro*-1,3-*dihydroxy*-6-*methyl*-2-*oxo*-1,4,5-*triazanaphthalene* (VI; R=Me, X–Y = N=C(OH)). Reduction of 2-ethoxalylamino-6-methyl-3-nitropyridine in sodium hydroxide solution (30 min) yielded, on concentration, the *triazanaphthalene* (38%) as colourless needles (from ethanol), m.p. 252–253° (decomp.). Found: C, 50·25; H, 3·7; N, 22·0. $C_8H_7N_3O_3$ requires C, 49·7; H, 3·6; N, 21·8%. ν_{max} 2,500–3,200 w (O–H), 1,680 s and 1,720 s cm^{-1} (C=O).

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References

Adachi, K. (1957). *Yakugaku Zasshi*, 77, 507–510.
Albert, A. & Barlin, G. B. (1963). *J. chem. Soc.*, 5737–5741.
Coutts, R. T. & Wibberley, D. G. (1963). *Ibid.*, 4610–4612.
Crowther, A. F., Curd, F. H. S., Davey, D. G. & Stacey, G. J. (1949). *Ibid.*, 1260–1271.
Dunn, G., Gallagher, J., Newbold, G. T. & Spring, F. S. (1949). *Ibid.*, S 126–131.
Dutcher, J. D. (1947). *J. biol. Chem.*, **171**, 321–339.
Elina, A. S. (1962). *Zhur. obshchei Khim.*, **32**, 2967–2973.
Honkanen, E. & Virtanen, A. I. (1960). *Acta. chem. scand.*, **14** (No. 5), 1214–1217.
Newbold, G. T. & Spring, F. S. (1948). *J. chem. Soc.*, 1864–1866.
Ochiai, E. & Ohta, A. (1962). *Chem. Pharm. Bull. Japan*, **10**, 1260–1261.
Tennant, G. (1963). *J. chem. Soc.*, 2428–2433.
White, E. C. (1940). *Science*, **92**, 127.
White, E. C. & Hill, J. H. (1943). *J. Bact.*, **45**, 433–442.