TABLE II
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE COMPOUNDS TESTED[a]

Microorganism	1	3	4	5	6	8	9	11	12	13
Neisseria catharrhalis						+				
Salmonella paratyphi B									+	
Klebsiella sp.	+									
Pseudomonas aeruginosa										+
Candida albicans					+	+				
Cryptoccocus neoformans										+
Nocardia asteroides								+		
Aspergillus fumigatus	+									
Trichophyton rubrum	+	+	+	+			+			
Trichophyton schoenleini	+			+						
Trichophyton mentagrophytes										+
Histoplasma capsulatum	+									

[a] + means a total inhibition of microbial growth.

required for the preparation of **11**, was obtained employing the procedure described for analogous compound.[12]

Preparation of Compounds 1–11.—Thenoyl chloride (3.66 g, 0.024 mole) was added dropwise during 0.5 hr to a well-stirred and ice-cooled solution of 0.02 mole of the particular amine in 20 ml of pyridine. To complete the reaction in the case of **1, 2, 5, 6,** and **10,** the reaction mixture was stirred for 2 hr at room temperature. To prepare **3, 4, 7–9,** and **11** the mixture was refluxed for 6 hr. After cooling overnight, **9** and **10** separated. Crude products were collected on a filter, washed with dilute HCl (*ca.* 3%), and recrystallized. Other products crystallized on pouring the reaction mixture on ice and were filtered off, washed with dilute HCl, and recrystallized.

2-(2-Thenoylamino)-5-chlorobenzophenone Hydrazone (12).—Compound **10** (6.7 g, 0.025 mole) and 0.81 g (0.025 mole) of hydrazine in 15 ml of EtOH were placed in a sealed tube and heated for 5 hr at 150°. The reaction mixture was cooled and poured on ice. The crude product which separated was filtered off, dried (6.3 g, 70%), and recrystallized as indicated in Table I.

2-(2-Thenyl)-4-phenyl-6-chloroquinazoline (13).—Compound **10** (2 g, 0.0076 mole) in 13 ml of 6.5% NH_3 solution in EtOH was sealed in a glass tube and heated for 5 hr at 140°. On cooling the crude product which separated was collected on a filter, washed (EtOH), and recrystallized.

(12) J. F. J. Dippy and V. Mass, *J. Chem. Soc.*, 2205 (1952).

Synthesis of 5,8-Quinazolinedione

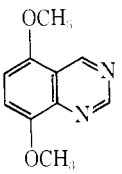
GIORGIO MALESANI, FRANCESCO MARCOLIN, AND GIOVANNI RODIGHIERO

Institute of Pharmaceutical Chemistry of the University of Padua, Padua, Italy

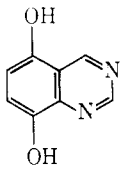
Received July 28, 1969

Quinones containing heterocyclic rings appear to be interesting from various points of view. Of a large number of 4,7-indolequinone derivatives related to mytomicin antibiotics,[1] several had interesting antibacterial activities. Various 5,8-quinolinediones were studied in relation to their antibacterial and cytostatic activities.[2] Recently also 5,8-isoquinolinedione[3] and some 5,8-quinoxalinedione derivatives have been prepared and studied. We were therefore led to investigate the closely related 5,8-quinazolinedione.

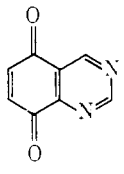
We recently prepared 5,8-dimethoxyquinazoline (**1**) and some derivatives,[5] for instance, 5-methoxy-8-hydroxyquinazoline. This compound was found to have antibacterial properties analogous to those of 8-hydroxyquinoline.



1



2



3

The complete demethylation of **1** was achieved by heating the substance with $AlCl_3$ at 180°. 5,8-Quinazolinedione (**3**) was prepared by $K_2Cr_2O_7$ oxidation. It was stable under normal conditions of storage and displayed typical quinonic behavior to KI–H_2SO_4, diphenylbenzidine–H_2SO_4,[6a] and ethyl cyanoacetate–alcohol NH_3[6b] test reagents. Mixed with 5,8-dihydroxyquinazoline it easily formed the quinhydrone derivative.

Biological Results[7]—5,8-Dihydroxyquinazoline (**2**) was tested on three strains of *Staphylococcus aureus* (I 67, Pd 2, Ba 61) and on *Streptococcus pyogenes* (N.T.C.C.S.T.A.), both in the absence and in the presence of equal molar amounts of Fe^{3+}. 5,8-Quinazolinedione (**3**) was tested on the same strains of *S. aureus* and of *S. pyogenes* and on *Escherichia coli* (Pd 3), *Salmonella typhi* (murium), and *Candida albicans*. Neither **2** nor **3** exhibited antibacterial activity below a concentration of 100 μg/ml.

5,8-Dihydroxyquinazoline (**2**) has a structure analogous to that of 8-hydroxyquinoline; the antibacterial activity of this substance is related to complex formation with various transition metal ions.[8] The ineffective antimicrobial activity of **2**, which does form stable metal ion complexes, may be attributable to its low partition coefficient in oleyl alcohol–H_2O (0.33). This factor in many cases can be correlated with the antibacterial activity of 8-hydroxyquinoline derivatives.[9a,b]

(1) M. J. Weiss, G. S. Redin, G. R. Allen, Jr., A. C. Dornbush, H. L. Lindsay, J. F. Poletto, W. A. Remers, R. H. Roth, and A. E. Sloboda, *J. Med. Chem.*, **11**, 742 (1968).

(2) (a) S. Petersen, W. Gauss, and E. Urbschat, *Angew. Chem.*, **67**, 217 (1955); (b) C. A. Schellhammer, S. Petersen, H. B. König, and G. Domagk, *Naturwissenschaften*, **46**, 82 (1959).

(3) (a) P. K. Joseph and M. M. Joullié, *J. Med. Chem.*, **7**, 801 (1964); (b) M. Lora-Tamayo, R. Madroñero, and M. Stud, *Chem. Ber.*, **95**, 2176 (1962).

(4) (a) M. R. W. Levy and M. M. Joullié, *J. Heterocyclic Chem.*, **1**, 171 (1964); **3**, 529 (1966); (b) W. F. Gum, Jr., and M. M. Joullié, *J. Org. Chem.*, **32**, 53 (1967).

(5) G. Malesani, A. Pietrogrande, and G. Rodighiero, *Farmaco Ed. Sci.*, **23**, 765 (1968).

(6) (a) V. Anger, *Mikrochim. Acta*, 386 (1959); (b) R. Craven, *J. Chem. Soc.*, 1605 (1931).

(7) The tests for antimicrobial activity were carried out by Dr. P. Benetti in the Institute of Health of the University of Ferrara, and those for antitumor activity by Dr. F. Baccichetti in this institute.

(8) (a) S. D. Rubbo, A. Albert, and M. J. Gibson, *Brit. J. Exp. Pathol.*, **31**, 425 (1950); (b) A. Albert, M. J. Gibson and S. D. Rubbo, *ibid.*, **34**, 119 (1953).

(9) (a) A. Albert and A. Hampton, *J. Chem. Soc.*, 505 (1954); (b) A. Albert, A. Hampton, F. R. Selbie, and R. D. Simon, *Brit. J. Exp. Pathol.*, **35**, 75 (1954); (c) A. Albert, *Biochem. J.*, **47**, 531 (1950); (d) H. Irving, and H. S. Rossotti, *J. Chem. Soc.*, 2910 (1954).

Compound **3** displayed practically no antitumor activity against Sarcoma 180 and mouse Ehrlich ascites; its acute LD_{50} on mice was 8.4 mg/kg ip.

Experimental Section

Melting points are uncorrected. Microanalyses are indicated only by symbols of the elements; unless otherwise stated, analytical results were within ±0.4% of the theoretical values. The uv absorption spectra were measured on an Optica CF 4 spectrometer. Ir spectra were recorded on a Perkin-Elmer 21 spectrometer.

5,8-Dihydroxyquinazoline (2).—A mixture of 4.5 g (23.7 mmoles) of 5,8-dimethoxyquinazoline (**1**) and 20 g (150 mmoles) of anhydrous $AlCl_3$ was heated in an oil bath at 170–180° for 8 hr. The reaction mixture was dissolved in 200 ml of H_2O and the solution was extracted with Et_2O (six 500-ml portions). The combined yellow extracts, which were dried (Na_2SO_4) and distilled at atmospheric pressure, yielded 1.08 g of a yellow solid which was sublimed *in vacuo* (0.001 mm). The fraction which sublimed below 140° was discarded, while the crystalline product which sublimed between 160 and 170° was crystallized from EtOAc; yield 0.48 g (12.5%) of yellow needles: mp 253°; λ_{max}^{EtOH} 205 nm (log ϵ 4.21), 249 (4.46), 340 (3.49); ν_{max} (KBr) 3455 (OH) and 1028 cm^{-1}. *Anal.* ($C_8H_6N_2O_2$) C, H, N.

Physical Measurements.—The acid ionization constant of **2** was determined by potentiometric titration;[10] pK_a (acid) at 20°, 8.4. The stability constants of metal complexes of **2** were determined by potentiometric titrations[9a,c,d] with Cu^{2+}, log $K' = 9.8$; with Co^{2+}, log $K' = 8.0$.

The partition coefficient in oleyl alcohol–H_2O was determined according to the method of Albert and Hampton:[9a] at 20°, the value is 0.33.

5,8-Quinazolinedione (3).—To an ice-cold stirred solution of 0.5 g (3.1 mmoles) of 5,8-dihydroxyquinazoline (**2**) in 50 ml of 10% H_2SO_4 was added a solution of 0.35 g (1.2 mmoles) of $K_2Cr_2O_7$ in 6 ml of H_2O. The solution was stirred with cooling (ice bath) for 45 min and extracted with $CHCl_3$ (five 300-ml portions). After distillation of the solvent at atmospheric pressure, the residue, crystallized three times from C_6H_6–petroleum ether (bp 30–50°) (1:1) yielded 0.37 g (74.9%) of a crystalline yellow-brown substance which decomposed, without melting, above 350°; λ_{max}^{EtOH} 205 nm (log ϵ 4.21), 249 (4.33), 325 (3.40), 341 (3.49); ν_{max} 1678 (C=O), 1575 cm^{-1}. *Anal.* ($C_8H_4N_2O_2$) C, H, N.

The quinhydrone of 2 and 3 was prepared by mixing separate solutions containing 25 mg each of **2** and **3** dissolved in 5 ml of PhMe. After standing in the cold, red-brown crystals formed, mp 318°. *Anal.* ($C_{16}H_{10}N_2O_4$) H, N; C: calcd, 59.63; found, 58.83.

Acknowledgment.—These studies were supported by the financial aid of Consiglio Nazionale delle Ricerche, Rome.

(10) (a) A. Albert, D. Brown and G. Cheeseman, *ibid.*, 474 (1951); (b) A. R. Osborn and K. Schofield, *ibid.*, 4191 (1956).

Terpene Compounds as Drugs. VII. Terpenylhydroxamic Acids

SILVANO CASADIO, ANTONIO MANTEGANI, AMEDEO OMODEI SALE', AND GERMANO COPPI

Research Laboratories of Istituto De Angeli, 20139 Milan, Italy

Received July 8, 1969

Hydroxamic acids possess biologic effects which include a particularly valuable antifungal activity.[1] As part of our program in the field of terpene compounds, we have prepared a series of terpenylhydroxamic acids and tested their antifungal properties. A report[2] on a possible therapeutic application of the urease inhibitory effect of hydroxamic acids[3] prompted us to test our compounds for this activity as well. The compounds were prepared by reaction of NH_2OH with the appropriate carboxylic ester; their chemical data are listed in Table I.

Antifungal activity was evaluated against four fungi according to a method previously described;[4] for comparative purposes 10-undecenohydroxamic acid (**10**) and nystatin were assayed concurrently. The results, reported in Table II, indicate that only compounds derived from sesquiterpenes displayed interesting antifungal activity; among them **6**, which proved as active as nystatin, appears to be worthy of a more detailed study. The inhibitory effect of terpenylhydroxamic acids on bacterial urease *in vitro* was tested, in comparison with acetohydroxamic acid, according to a new procedure.[5] The enzyme was incubated at 37° in a solution of urea in phosphate buffer with addition of the test compound. After 20 and 30 min, NH_3 liberated by the enzyme was assayed according to the method of McCullough.[6] The inhibitions, reported in Table III, were calculated for control tests performed without any addition of compounds. Potency of **3** and **9** *in vitro* was comparable with that of acetohydroxamic acid; **4** was less active, whereas other compounds were inactive. Compounds **3**, **4**, **9**, and acetohydroxamic acid were tested on hyperammonemia induced by intraperitoneal injections of urea (200 mg/kg) and urease (25 mg/kg) in rats.[5] Acetohydroxamic acid, at a dose of 100 mg/kg orally, significantly reduced blood NH_3 2, 4, 6, and 8 hr after urea–urease injections; compounds **3**, **4**, and **9**, tested at the same dose, exhibited no activity.

Experimental Section[7]

Method A. Geranoylhydroxamic Acid (1).—A solution of NaOH (12.4 g, 0.36 mole) in 50% MeOH (50 ml) was added at 10–15° with stirring under N_2 to $NH_2OH \cdot HCl$ (18.1 g, 0.26 mole) dissolved in H_2O (23 ml). Methyl geranate (36.5 g, 0.2 mole) was subsequently added and the mixture was stirred for 6 hr at room temperature. Acidification to pH 2–3 with 15% HCl and evaporation of MeOH at reduced pressure gave a suspension which was extracted with Et_2O. The Et_2O layer was extracted with 3% NaOH and the alkaline solution was acidified with 15% HCl to give an oil which was extracted with Et_2O and dried ($MgSO_4$). Evaporation of the solvent gave a residue (10.2 g) of crude **1**. This product, dissolved in AcOH (15 ml), was dropped with vigorous stirring into a solution of copper acetate (16.6 g, 0.083 mole) in H_2O (230 ml). The green gummy precipitate was thoroughly washed (H_2O, absolute EtOH), filtered, and dried. The solid obtained was then shaken with Et_2O (300 ml) and 25% H_2SO_4 (100 ml) to complete dissolution. The Et_2O layer, washed (H_2O) and dried (Na_2SO_4), was evaporated to give 5.5 g of **1** as a colorless oil.

Method B. Citronelloylhydroxamic Acid (2).—Crude citronelloylhydroxamic acid (prepared according to method A) was taken up in petroleum ether (bp 40–70°) and allowed to stand in an

(1) (a) H. Kitagawa, S. Yoshida, M. Abe, W. C. Chen, and T. Arai, *J. Pharm. Soc. Jap.*, **85**, 860 (1965); (b) R. M. Patel, D. P. Carew, and J. L. Lach, *J. Pharm. Sci.*, **56**, 1326 (1967).

(2) W. N. Fishbein, P. P. Carbone, and H. D. Hochstein, *Nature*, **208**, 46 (1965).

(3) K. Kobashi, J. Hase, and K. Uehara, *Biochim. Biophys. Acta*, **65**, 380 (1962).

(4) G. Coppi, A. Maselli, and C. Ciani-Bonardi, *Farmaco, Ed. Sci.*, **20**, 203 (1965).

(5) G. Coppi and G. Bonardi, manuscript in preparation.

(6) H. McCullough, *Clin. Chim. Acta*, **17**, 297 (1967).

(7) Melting points are corrected and were taken on a Büchi capillary melting point apparatus. Purity of compounds was checked by tlc, ir, and nmr.