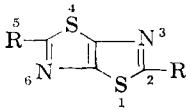
Derivatives of Thiazolo[5,4-d]thiazole

By HIKMAT T. FIKRAT and J. F. ONETO

A series of bis-2,5-substituted thiazolo[5,4-d]thiazoles has been prepared and examined for biological activity.

THE CONDENSATION of dithio-oxamide with aromatic aldehydes was first described by Ephraim (1). Recently, Johnson and Ketcham (2) reported on studies concerning this reaction and established the structure of the resulting parent heterocycle as thiazolo[5,4-d]thiazole, I, with substituents at the two and five positions being equivalent.



I

The purpose of our investigation was to prepare a number of hitherto undescribed thiazolothiazole derivatives for biological examination. Compounds of this class were considered to be of empirical interest because of the included thiazole moiety which occurs in a wide variety of agents exhibiting diverse biological activities.

The synthesis was effected in low to moderate yields (10–88%) through condensation of dithio-oxamide with appropriate aldehydes following essentially the published procedure (2). Of particular interest was the development of an alternate preparative method which was studied in the preparation of the previously described benzaldehyde derivative (2), and the pyridine-3-aldehyde and 2,2-dimethyl-4-cyanobutyraldehyde derivatives (Table I). The condensation was carried out in refluxing pyridine and indicated that in the above instances high reaction temperatures were not essential for successful results. However, the yields obtained by this approach were invariably lower than those realized from the published procedure.

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The apparent limitation of the reaction to aromatic type aldehydes has been reported (2). Our investigation of a number of saturated and α,β-unsaturated aliphatic aldehydes, in addition to the aldehydes studies by Johnson and Ketcham, supports this observation with the exception of the previously mentioned 2,2-dimethyl-4-cyanobutyraldehyde. As this cyano aliphatic aldehyde is devoid of α hydrogen, it was of interest to investigate the reaction behavior of the analogous hydroxypivaldehyde-(2,2-dimethyl-3-hydroxypropionaldehyde) and its acetyl and benzoyl derivatives. Efforts to obtain the corresponding thiazolothiazole derivatives from these aldehydes were unsuccessful.

The reaction of quinoline-2-aldehyde with dithio-oxamide yielded an acid-soluble compound melting at 109–111°, and an acid-insoluble compound which melted over a wide range (230–265°) after several recrystallizations from chloroform. Neither of the products proved to be thiazolothiazole derivatives.

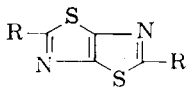
During the course of the investigation, the diamide, dihydrazide, and diisopropylidenehydrazide derivatives of thiazolothiazole-2,5-dicarboxylic acid were prepared and studied.

Inasmuch as bis-(1,1-dimethyl-3-cyanopropyl)-thiazolo[5,4-d]thiazole is the only compound to have been prepared from an aliphatic aldehyde, its structure in addition to analyses was further confirmed by comparing its ultraviolet and infrared spectra with those reported for the parent heterocycle (3).

The ultraviolet absorption of the compound was measured with a Cary model 11 spectrometer at a concentration of 2.5 mg. % in 95% ethanol. Bands appearing at 244 mμ (log ϵ = 3.85) and 251 mμ (log ϵ = 4.11) correspond in shape and contour with the reported thiazolothiazole bands at 242 and 255 mμ (log ϵ = 3.94). A strong band appearing at 278 mμ corresponds with the 260 mμ (log ϵ = 3.87) of thiazolothiazole.

The infrared absorption of the compound was measured in a potassium bromide disk (3 mg. per 400 mg. KBr) with a Perkin-Elmer model 21 spectrophotometer. The observed characteristic vibrational frequencies lead to the following assignments: a broad unresolved band at 3.41 μ in the C—H stretching region is understandable due to the increased variety of C—H bonds as compared with the sharp 3.31 μ band due to the

TABLE I.—BIS-2,5-SUBSTITUTED THIAZOLO[5,4-d]THIAZOLES



No.[a]	R	R—CHO Ratio[b]	Time,[c] Min.	Intern. Temp.,[c] °C.	Yield, %	M.p.,[d] °C.	Formula	Analyses,[e] % Nitrogen Calcd.	Nitrogen Found	Sulfur Calcd.	Sulfur Found
1	2-Pyridyl	8	30	160–170	51	325–326	$C_{14}H_8N_4S_2$	...	...	21.63	21.46
2	3-Pyridyl	8	30	165–175	59	268–269	$C_{14}H_8N_4S_2$	18.95	19.11	...	...
		(8)			(38)[f]						
3	4-Pyridyl	8	30	160–165	85	300–302	$C_{14}H_8N_4S_2$	18.95	19.07	...	...
4	6-Methyl-2-pyridyl	10	20	170–200	56	299–302	$C_{16}H_{12}N_4S_2$	17.26	17.50	19.77	19.48
5	2-Carboxyphenyl	10	15	220–230	10	302–305	$C_{18}H_{10}N_2O_4S_2$	7.32	7.48	16.79	16.52
6	2,4-Dimethoxyphenyl	5	25	180–200	58	291–293	$C_{20}H_{18}N_2O_4S_2$	6.76	6.94	15.47	15.45
7	3,4-Dimethoxyphenyl	8	30	195–200	64	269–272	$C_{20}H_{18}N_2O_4S_2$	6.76	6.74	15.47	15.25
8	3-Methoxy-4-hydroxyphenyl	8	30	180–200	52	270–273	$C_{18}H_{14}N_2O_4S_2$	7.32	7.47	16.77	16.56
9	4-Acetamidophenyl[g]	7.5	5	190–200	73	388–396	...	...	...	...	...
10	2-Hydroxy-1-naphthyl	5	20	170–200	54	338–342	$C_{24}H_{14}N_2O_2S_2$	6.57	6.72	15.04	14.86
11	2-Thienyl	5	20	170–180	88	238–240	$C_{12}H_6N_2S_4$	9.14	9.05	41.85	41.62
12	3-Indolyl	5	30	175–180	31	>400	$C_{20}H_{12}N_4S_2$	15.07	15.32	17.22	17.01
13	4-Quinolyl	10	5[h]	230	40	254–256	$C_{22}H_{12}N_4S_2$	14.14	13.85	...	...
14	1,1-Dimethyl-3-cyanopropyl[i]	5	20	200	39	109–111	$C_{16}H_{20}N_4S_2$	...	...	19.29	19.09
		(8)			(27)[f]						

[a] Compounds 1, 2, 3, 4, 7, and 12 were recrystallized from pyridine; 5 from ethanol; 6 and 8 from phenol-ethanol; 10 from pyridine or benzene-alcohol; 11 from pyridine, chloroform or benzene; 13 from chloroform, and 14 from acetone, benzene, ethanol, or ether. [b] Molar ratio of aldehyde to dithio-oxamide. [c] Time of heating during the indicated internal temperature range. [d] Melting points were determined on a Nalge m.p. apparatus and are uncorrected. [e] Analyses were performed at the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. [f] Procedure B. [g] The crude product was hydrolyzed with hydrochloric acid in butanol to the bis-4-aminophenyl derivative; m.p. 296–300° [Lit. (2) 301–303°]. [h] The reaction mixture was then cooled to 180–200° and maintained at that temperature range for 20 minutes. [i] The 2,2-dimethyl-4-cyanobutyraldehyde was supplied through the courtesy of Eastman Chemical Products, Inc., Kingsport, Tenn.

C—H stretch in thiazolothiazole. The presence of the nitrile group is indicated by a characteristic stretching band at 4.5 μ. Peaks at 6.7, 6.8, and 6.9 μ can be assigned to the $-CH_2-$ and $-CH_3$ deformation vibrations. Peaks at 7.14 and 7.26 μ are assigned to the deformation vibrations of the $=C(CH_3)_2$ grouping. The assigned structure appears to be in general agreement with the infrared spectrum.

BIOLOGICAL RESULTS

The compounds appearing in Table I, with the exception of compound 9, were screened at the Parke Davis and Co. Laboratories for antibacterial activity. In addition, the screening program included bis-(4-aminophenyl)thiazolothiazole and thiazolothiazole-2,5-dicarboxylic acid and its dihydrazide and diisopropylidenehydrazide derivatives.

The compounds were tested against representative strains of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Mycobacterium tuberculosis*. None of the compounds showed antibacterial activity.

Compound 7 and thiazolothiazole-2,5-dicarboxylic acid were tested for antiviral activity with negative results.

Compounds 1, 2, 3, 4, and 14 showed no effect when tested for amebacidal activity.

Bis-(4-aminophenyl)-thiazolothiazole produced a 20% increase in blood cholesterol when administered to rats by intubation at a 5-mg. daily dose level for 7 days.

Compound 14, the 1,1-dimethyl-3-cyanopropyl derivative, was subjected to a preliminary pharmacological screening and appears to differ in its action from the usual central nervous system depressants.

The acute LD_{50} determined in mice is greater than 1250 mg./Kg. subcutaneously.

When given to dogs, rats, mice, and guinea pigs, orally or intramuscularly in doses of 2–200 mg./Kg., the compound induced normal sleep; that is, animals could be awakened by stimulation, but would subsequently return to sleep even in the laboratory environment. No excitement, respiratory depression, or general anesthesia was seen. Onset of action was 15–30 minutes; duration 2–3 hours. The electroencephalogram showed an ordinary sleep pattern during the action of the drug. The compound appeared qualitatively similar to trimeglamide (4) when directly compared.

The effect of sodium pentobarbital was intensified by the simultaneous administration of compound 14 whether measured by prolongation of sleeping time after anesthetic doses of pentobarbital or augmentation of the effect of subanesthetic doses. The tested compound was given in doses of 25–200 mg./Kg. intraperitoneally.

No autonomic or cardiovascular effects were demonstrable in anesthetized dogs with the smaller (50 mg./Kg.) doses tolerated by the unanesthetized animals.

No analgetic effect, strychnine antagonism, or protection against apomorphine emesis were demonstrable. Minor protection against pentylenetetrazol convulsions was shown.

The compound appears to have highly selective effects on the central nervous system, manifested almost purely by induction of sleep. However, it differs from the sedative-hypnotics in that an ex-

citement is not seen nor can anesthesia and medullary depression be induced by increasing the dose. Unlike the tranquilizers, this thiazolothiazole derivative does not alter autonomic function or exhibit evidences of central stimulation.

EXPERIMENTAL

General Procedures.—The molar ratio of reactants and other reaction data appear in Table I. *Procedure A.*—Following the published procedure (2), a mixture of dithio-oxamide and the aldehyde was heated under a stream of nitrogen in a wax bath. The reaction mixture was cooled and treated with ethanol. The product was filtered and recrystallized.

Procedure B.—Procedure A was modified by refluxing the reactants in dry pyridine for 60 hours. After cooling, the product was filtered and recrystallized.

Dihydrazide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—A mixture of 0.5 Gm. (2 mmoles) of bis-(carbomethoxy)-thiazolothiazole, prepared from the corresponding acid (2) and diazomethane, 325 mg. (6 mmoles) of hydrazine hydrate (85% aqueous solution) and 125 ml. of ethanol was refluxed for 6 to 7 hours. The reaction mixture was concentrated by distillation under reduced pressure, filtered, and the product washed with ethanol. The yield was practically quantitative. A suitable recrystallization procedure was not achieved. The crude washed product, m.p. > 400°, gave the following analytical results.

Anal.—Calcd. for $C_6H_6N_6O_2S_2$: N, 32.56; S, 24.84. Found: N, 31.20; S, 24.78.

Diisopropylidenehydrazide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—Five drops of glacial acetic acid was added to a suspension of 0.5 Gm. (2 mmoles) of the dihydrazide of thiazolothiazole dicarboxylic acid in 100 ml. of reagent acetone. After refluxing for 1 hour, the reaction mixture was concentrated under reduced pressure and filtered. The yield was practically quantitative. After recrystallization from acetone-ethanol, the product melted at 296–298°.

Anal.—Calcd. for $C_{12}H_{14}N_6O_2S_2$: N, 24.84; S, 18.95. Found: N, 25.28; S, 18.60.

Diamide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—A fine suspension of bis-(carbomethoxy)-thiazolothiazole was prepared by dissolving 0.2 Gm. (0.78 mmole) of the ester in 5 ml. of boiling dioxane, followed by rapid cooling in an ice bath. Five milliliters of ammonium hydroxide (27%) was added and the mixture allowed to stand overnight at room temperature. After concentrating the mixture to one-half volume under reduced pressure, the white precipitate was filtered and washed with water and ethanol. The yield was practically quantitative. A suitable recrystallization procedure was not achieved. The crude, washed product did not melt when heated to 400°.

Anal.—Calcd. for $C_6H_4N_4O_2S_2$: N, 24.55; S, 28.00. Found: N, 24.30; S, 27.86.

REFERENCES

(1) Ephraim, J., *Ber.*, **24**, 1027(1891).
(2) Johnson, J. R., and Ketcham, R., *J. Am. Chem. Soc.*, **82**, 2719(1960).
(3) Ketcham, R., thesis, Doctor of Philosophy, Cornell University, 1956.
(4) Cronheim, G., Gourzis, J. T., and Toekes, I. M., *Science*, **128**, 1570(1958).

Pantothenyl Alcohol Effect on Delta-1-Cortisol-Induced Gastric Ulcers

By THOMAS A. LYNCH, WILBUR L. HIGHLEY, and ALBERT G. WORTON

The development of a standardized technique in assessing the ulcerogenic side effects of the corticosteroid drugs has provided an effective means of evaluating antiulcer agents. Approximately 90% incidence of gastric ulcers can be produced in fasting female rats by daily subcutaneous administration of 40 mg./Kg. delta-1-cortisol (D1C) for 4 days. Pantothenyl alcohol, extensively used in treating postoperative intestinal atony, was evaluated for antiulcer activity in such treated animals by two methods: (*a*) oral feeding during the 4 days induction period to determine effect on development on ulcers and (*b*) oral feeding after induction of ulcers to determine the effects on spontaneous healing. Pantothenyl alcohol did not reduce the degree of ulceration during the induction period, but markedly accelerated spontaneous ulcer healing.

THE IMPORTANCE of pantothenic acid to gastrointestinal integrity has been demonstrated by studies involving pantothenic acid deprivation and the pituitary axis. In 1943, Bly, *et al.* (1), demonstrated a 50% reduction of intestinal motility in pantothenic acid-deficient dogs. About the same time, Jurgens and Pfaltz (2) observed atony and distention of the gastrointestinal tract of pantothenic acid-deficient rats. These observations helped establish the basis for the use of pantothenic acid in the treatment of postoperative intestinal atony and gaseous intestinal distention (3–6). The discovery by Lipmann, *et al.* (7), that pantothenic acid formed an integral part of coenzyme A, and the pantothenic deficiency studies in humans by Bean,

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