TABLE II

ORAL PROGESTATIONAL ACTIVITY OF KETALS AND CORRESPONDING KETONES

Compd.	Relative potency[a]	Endometrial response	Total dose, mg.
17-Acetoxyprogesterone	1	3.0	20
		2.3	10
		1.5	5
3-ethylene ketal[3]	3		
6α-Methyl-17-acetoxyprogesterone[5]	60	1.6	0.1
		3.6	0.4
3-ethylene ketal		2.2	0.1
		2.5	0.2
		3.0	0.4
3-(1,2-dimethylethylene) ketal		1.2	0.1
6-Dehydro-17-acetoxyprogesterone[8]	26		
3-ethylene ketal	25		
6-Dehydro-6-methyl-17-acetoxyprogesterone[8]	200		
3-ethylene ketal	114		
6-Chloro-6-dehydro-17-acetoxyprogesterone[9]	190	3.1	0.08
3-ethylene ketal	320		
3-methylethylene ketal	430		
3-(1,2-dimethylethylene) ketal	520		
3-ethylenethio ketal		0	0.08
17-Ethylprogesterone[4,10]	0.5		
3-ethylene ketal	2		
		0	1.0 s.c.
		0	3.0 s.c.
17-Propylprogesterone[1,10]	2		
3-ethylene ketal[1]	3		
6-Dehydro-17-ethylprogesterone[10]	5	0.5	0.5
		1.2	1.0
		3.7	5.0
3-ethylene ketal		1.5	0.5
		1.8	1.0
		1.2	2.0
		3.8	5.0
		3.8	0.5 s.c.
		4.0	1.5 s.c.
6-Chloro-6-dehydro-17-ethylprogesterone[10]	60	0.5	0.04
		2.6	0.16
3-ethylene ketal	52		
3-(1,2-dimethylethylene) ketal		0.2	0.04
		3.2	0.16

[a] All values are based on data obtained by the present authors.[14] Relative potencies were determined by plotting dose–response data on semilog paper. Where a dose–response effect was not observed or where a complete evaluation was not obtained, the activity is indicated in terms of the endometrial response to a given total dose (mg.). This response (or lack thereof) is measured in terms of 0 to 4, the latter number indicating the highest activity.

2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 100 ml. of dioxane saturated with HCl. The product was recrystallized from acetone–hexane to give 1.922 g. (50%) of white crystals, m.p. 213–215° (lit.[8b] m.p. 218–220°), $[\alpha]^{25}D$ +19° (*c* 1.0, $CHCl_3$) (lit.[8b] $[\alpha]D$ +11°, $CHCl_3$), $\lambda_{max}^{CH_3OH}$ 289 mμ (ε 24,000).

General Ketalization Procedure.—The following preparation of **17-acetoxy-3-ethylenedioxypregna-4,6-dien-20-one** is illustrative. A solution of 400 mg. of 17-acetoxypregna-4,6-diene-3,20-dione,[6] 20 mg. of *p*-toluenesulfonic acid and 20 ml. of ethylene glycol in 100 ml. of reagent grade benzene was stirred vigorously at reflux for 5 hr. The water formed was removed by means of a Dean–Stark tube. The cooled solution was poured into 100 ml. of 5% aqueous sodium carbonate solution. The organic phase was separated, diluted with ether, washed with saline and water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The resulting solid was triturated with ether and collected to give 346 mg. (77%) of product, m.p. 220–225° (see Table I). The various 3-ketals prepared by this procedure are listed in Table I.

17-Acetoxy-6-chloro-3-ethylenedithiopregna-4,6-dien-20-one.[15]—A mixture containing 95 mg. of 17-acetoxy-6-chloropregna-4,6-diene-3,20-dione,[9] 2 ml. of acetic acid, 26 mg. of 1,2-ethanedithiol, and 30 mg. of *p*-toluenesulfonic acid was kept at room temperature for 1 hr. and then was poured into water with stirring. The precipitated solids were filtered, washed well with water, and dissolved in chloroform. After drying over anhydrous sodium sulfate, the chloroform solution was evaporated to dryness to give the product (see Table I).

Stability of 17-Acetoxy-6-chloro-3-ethylenedioxypregna-4,6-dien-20-one in Tragacanth.—Subject ketal (100 mg./ml.) was suspended in 10 ml. of tragacanth (pH 5.4, from the same source as that used for assay purposes). Aliquots of this suspension were taken 0, 3, and 5 days after preparation. These aliquots were extracted three times with equal volumes of chloroform, the total volume was adjusted to 50 ml., and the ultraviolet spectrum was determined. In all instances approximately 95% of steroid could be accounted for as ketal, which was confirmed by paper chromatography with a methanol–water–heptane (4:1:5) system. Samples of the chloroform extract partitioned for 90 min. on Whatman paper No. 3 each exhibited a single ultraviolet-absorbing spot, with a degree of migration identical with that of concurrently partitioned standard ketal and different from that of parent ketone. (Parallel experiments with this ketone, 17-acetoxy-6-chloropregna-4,6-diene-3,20-dione showed that this ketone also is absorbed into the chloroform layer.)

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Studies on Methylglyoxal Bis(guanylhydrazone)[1] Analogs. III. Trifluoromethylglyoxal Bis(guanylhydrazone)[2] and 1,2-Bis(guanidinoamino)propane[3,4]

EUGENE G. PODREBARAC AND C. C. CHENG

Midwest Research Institute, Kansas City, Missouri 64110

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In view of the antileukemic activity of methylglyoxal bis(guanylhydrazone) (I) and the complete lack of activity of closely related homologs[5] and structural analogs,[6] it appears that steric factors play a

(1) According to *Chemical Abstracts*, the name for this compound is 1,1′-[(methyl)ethanediylidenedinitrilo]diguanidine.

(2) 1,1′-[(Trifluoromethyl)ethanediylidenedinitrilo]diguanidine.

(3) 1,1′-[(Methylethylene)diimino]diguanidine.

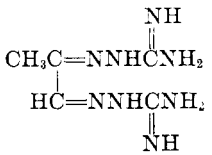
(4) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract SA-43-ph-3025.

(5) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *J. Med. Chem.*, **6**, 283 (1963).

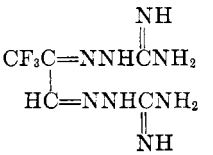
(6) (a) F. Baiocchi, C. C. Cheng, W. J. Haggerty, Jr., L. R. Lewis, T. K. Liao, W. H. Nyberg, D. E. O'Brien, and E. G. Podrebarac, *ibid.*, **6**, 431 (1963). (b) Butane-1,3-dione bis(guanylhydrazone) sulfate, originally reported by Burness,[7] was found to be inactive in L-1210 system.

(7) D. M. Burness, *J. Org. Chem.*, **21**, 97 (1956).

very important role in the structure–activity relationship of this type of compound. This is best illustrated by the fact that the replacement of the methyl group in I by an ethyl group resulted in the *total* loss of oncolytic activity. It was, therefore, desirable to synthesize trifluoromethylglyoxal bis(guanylhydrazone) (II) in order to better understand the relationship between the



I



II

electronic effect and biological activity in this type of structure. The close approximation of the size of a fluorine atom to that of a hydrogen atom keeps the difference of steric influence between compounds I and II to a minimum.

Since a number of alkylglyoxal bis(guanylhydrazones) were successfully prepared by the reaction of aminoguanidine with a dichloromethyl alkyl ketone,[5] an attempt was made to use this route for the preparation of II. McBee and Burton[8] reported the preparation of dibromomethyl trifluoromethyl ketone (III) by the bromination of trifluoroacetone in concentrated sulfuric acid. Treatment of III with 2 equiv. of aminoguanidine hydrobromide for an extended period gave a very low yield of II, isolated as the dihydrobromide

$CF_3COCHBr_2$	CF_3COCH_3	CF_3COCHO
III	IV	V

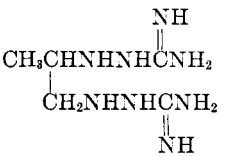
salt. The sulfate of II was similarly prepared from III and aminoguanidine sulfate. Both products were difficult to purify. The ultraviolet absorption maxima for the two were identical (λ_{max}^{pH1} 304 mμ), yet quite different from the characteristic absorption for I and its homologs (λ_{max}^{pH1} 283 mμ).[5]

In view of the analytical difficulties and the "abnormal" ultraviolet absorption, an attempt was made to determine whether the products in the foregoing experiments were actually bis(guanylhydrazones). Trifluoroacetone (IV) was oxidized with selenium dioxide. The general procedure of Riley and Gray[9] for the preparation of phenylglyoxal and that of Ronzio and Waugh[10] for the preparation of glyoxal was adapted for the preparation of trifluoromethylglyoxal. The aqueous solution of trifluoromethylglyoxal (V) thus obtained was treated *in situ* with 2 equiv. of aminoguanidine sulfate. The ultraviolet absorption spectrum of the solid product, which melted at 244–246° with decomposition, was identical with spectra of the previous runs.

A portion of the product was converted to the free base by a procedure similar to that described for the preparation of the free base of I.[6a] The n.m.r. comparison of the free bases of I and II further confirmed the structure of the product as II.[11] Paper chromatographic analyses of the free base and different salts of II indicated that all these products are fundamentally identical: R_f 0.63 for all four products in 5% ammonium bicarbonate (25°, descending) and 0.55 for all four products in ethanol–water–hydrochloric acid (8:1:1).

For purposes of comparison the guanylhydrazone of 1,1,1-trifluoroacetone was prepared from IV and aminoguanidine. The product, m.p. 190–191°, had an ultraviolet absorption maximum at 226 mμ (pH 1).

In addition, the closely related tetrahydro analog of I, 1,2-bis(guanidinoamino)propane (VI),[3] was prepared by catalytic hydrogenation of I.



VI

Preliminary antitumor evaluation of these compounds indicated that, at a dose of 75 mg./kg., trifluoromethylglyoxal bis(guanylhydrazone) sulfate monohydrate was not toxic and failed to inhibit the leukemia L-1210 tumor system.[12] By comparison, methglyoxal bis(guanylhydrazone) dihydrochloride is active against L-1210 at a dose of 20 mg./kg. and is toxic at 120 mg./kg. Consequently, testing at higher dosages for the trifluoromethyl analog is under way. The sulfate salt of 1,2-bis(guanidinoamino)propane (VI) is toxic at 500 mg./kg. in noninbred, Swiss albino mice and random-bred albino rats. It is inactive in sarcoma-180, leukemia L-1210, and Walker 256 (intramuscular) tumor systems at 125 mg./kg. In tissue culture studies compound VI has an ED_{50} (the dose that causes a 50% inhibition of growth) of 11 γ/ml. (slope, 0.52).[13]

Experimental[14]

Trifluoromethylglyoxal Bis(guanylhydrazone) Dihydrobromide.—To a solution of aminoguanidine hydrobromide [prepared from 38.0 g. (0.28 mole) of aminoguanidine bicarbonate and 47.1 g. (0.28 mole) of 48% hydrobromic acid] in 100 ml. of water was added 38.8 g. (0.14 mole) of dibromomethyl trifluoromethyl ketone.[8] The mixture was warmed on the steam bath for 8 hr. A solid slowly deposited when the reaction mixture was allowed to stand for a period of 4 weeks. The product, after recrystallization from a small amount of ethanol, weighed 8 g. When heated, it appeared to undergo a phase transition at 160–175°, giving a solid which decomposed at 235–237°, λ_{max}^{pH1} 304 mμ (ϵ 17,500), λ_{max}^{pH1} 348 mμ (ϵ 27,800).

Anal. Calcd. for $C_5H_9F_3N_8 \cdot 2HBr \cdot 1.5H_2O$: C, 14.1; H, 3.28; Br, 37.4; N, 26.3. Found: C, 14.1; H, 3.32; Br, 37.4; N, 26.3.

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(11) The spectra were run on a Varian A-60 high resolution n.m.r. spectrometer. The compounds were dissolved in trifluoroacetic acid using tetramethylsilane as an external standard (which introduced a deviation of ±0.2 p.p.m.). In the free base of methylglyoxal bis(guanylhydrazone) (I), the methyl protons appear as a single sharp peak at δ = 1.8 p.p.m., and the aldimine proton as a single sharp peak at δ = 7.4 p.p.m.; this is a normal chemical shift for the —CH=N— grouping. The N–H protons appear as a single broad band at δ = 6.4 p.p.m. In the case of the free base of trifluoromethylglyoxal bis(guanylhydrazone) (II), the methyl protons are absent and the aldimine proton appears at 7.6 p.p.m. The N–H protons appear as a double band, moderately mixed, but with separate maxima at δ = 6.7 and 6.9 p.p.m. The area of each band appears to be about the same but the downfield band (6.9 p.p.m.) is slightly larger.

(12) Dr. Richard H. Adamson, National Cancer Institute, private communication.

(13) Testing work was done by Microbiological Associates, a contract screener of Cancer Chemotherapy National Service Center.

(14) All melting points (corrected) were taken on a Thomas–Hoover melting-point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer. For carbon and hydrogen determinations of fluorine-containing compounds, magnesium oxide was added to combustion tubes; *cf.* A. Steyermark, "Quantitative Organic Micro-Analyses," 2nd Ed., Academic Press, New York, N. Y., 1961, p. 222.

Trifluoromethylglyoxal Bis(guanylhydrazone) Sulfate. A.—An aqueous solution of dibromomethyl trifluoromethyl ketone[8] and aminoguanidine sulfate[5] (in the ratio of 1:2) was refluxed for 5–15 hr. On cooling, the separated solid was filtered and recrystallized from water. Yields generally were in the range of 40–70%. The product, which decomposed at *ca.* 240° and had a characteristic ultraviolet absorption maximum of 304 mμ at pH 1, failed to yield a satisfactory analysis. (Typical analysis: C, 18.1; H, 3.78; N, 31.8.)

B.—A mixture of 111 g. of selenium dioxide, 600 ml. of dioxane, 20 ml. of glacial acetic acid, and 20 ml. of water was warmed on a steam bath for 3 hr. and cooled to room temperature. To the stirred suspension was added 112 g. of trifluoroacetone in one portion and the reaction mixture refluxed with stirring for 5 hr. The liquid was separated by filtration and the solid was washed with two 75-ml. portions of water. The combined filtrate and washings were distilled at atmospheric pressure to a volume of about 350 ml. The liquid was decanted from a slight amount of precipitated selenium and the volume was adjusted to about 500 ml. by addition of water. Lead acetate solution (25%) was added in slight excess. The lead selenite was removed by filtration and the filtrate was saturated with hydrogen sulfide to remove all traces of lead. Approximately 20 g. of activated charcoal was added. The mixture was warmed to about 40°, filtered with suction, and the colorless filtrate concentrated to about 300 ml. This concentrate was added dropwise to a 500-ml. stirred solution of 2 moles of aminoguanidine sulfate in water (prepared from 274 g. of aminoguanidine bicarbonate and 98 g. of sulfuric acid). The resulting turbid solution was refluxed for 3 hr. and stirred at room temperature for 48 hr. The yellow solid which separated (38 g.) decomposed at *ca.* 242°. Concentration of the filtrate yielded an additional 45 g. After recrystallization from water, it melted at 244–246° dec., λ_{max}^{pH1} 304 mμ (ε 16,600), λ_{max}^{pH11} 349 mμ (ε 26,900).

Anal. Calcd. for $C_5H_9F_3N_8 \cdot H_2SO_4 \cdot H_2O$: C, 17.0; H, 3.67; N, 31.6. Found: C, 17.3; H, 3.69; N, 31.9.

Trifluoromethylglyoxal Bis(guanylhydrazone) (II).—A suspension of trifluoromethylglyoxal bis(guanylhydrazone) sulfate in water was carefully neutralized with dilute sodium hydroxide at room temperature, and the resultant solution was extracted several times with butanol. The butanol extract was evaporated *in vacuo* to yield a yellow solid which, after recrystallization from a mixture of 2-propanol and heptane, gave II, m.p. 210° dec., λ_{max}^{pH1} 304 mμ (ε 18,700), λ_{max}^{pH11} 348 mμ (ε 21,400).

Anal. Calcd. for $C_5H_9F_3N_8$: C, 25.2; H, 3.78; N, 47.1. Found: C, 25.5; H, 4.20; N, 47.0.

1,1,1-Trifluoroacetone Guanylhydrazone Hemisulfate.—To a solution of aminoguanidine sulfate, prepared from 40.5 g. (0.30 mole) of aminoguanidine bicarbonate and 15.0 g. (0.153 mole) of sulfuric acid in 200 ml. of water was added at room temperature, 33.6 g. (0.30 mole) of 1,1,1-trifluoroacetone. The reaction mixture was stirred at room temperature for 2 hr., then warmed on a steam bath for 3 hr. Addition of approximately 20 ml. of absolute ethanol to the cooled solution caused immediate precipitation of a white solid which was isolated by filtration. The product (almost quantitative yield) was washed with a small quantity of cold absolute ethanol and dried, m.p. 190–191° (analyzed without further purification), λ_{max}^{pH1} 226 mμ (ε 15,800), λ_{max}^{pH11} 248 mμ (ε 15,900).

Anal. Calcd. for $C_4H_7F_3N_4 \cdot 0.5H_2SO_4$: C, 22.1; H, 3.69; N, 25.8. Found: C, 22.1; H, 4.10; N, 25.9.

1,2-Bis(guanidinoamino)propane Sulfate. A.—A suspension of 25.7 g. (0.1 mole) of methylglyoxal bis(guanylhydrazone) dihydrochloride monohydrate in 250 ml. of 60% acetic acid containing 0.1 g. of platinum oxide was hydrogenated at 4.22 kg./cm.^{2} for 24 hr. during which time the reaction vessel was intermittently warmed to about 50°. The theoretical amount of hydrogen was consumed. The catalyst was removed and the filtrate was evaporated *in vacuo* to give a very hygroscopic solid to which was added 100 ml. of water and 31.1 g. of silver sulfate. The mixture was shaken for 2 hr. and filtered to remove the silver chloride. Ethanol was added to the warmed filtrate until turbid, and the solution was allowed to cool slowly. The product, which failed to absorb in the ultraviolet region, was recrystallized from a mixture of water and methanol to give 10 g. of white solid, m.p. 290° dec.

Anal. Calcd. for $C_5H_{16}N_8 \cdot H_2SO_4$: C, 21.0; H, 6.30; N, 39.2. Found: C, 21.2; H, 6.76; N, 39.6.

B.—A suspension of 15 g. of methylglyoxal bis(guanylhydrazone) sulfate in 200 ml. of 50% acetic acid containing 1 g. of platinum oxide was hydrogenated at 65° and 4.22 kg./cm.^{2}. During 3 hr. the calculated amount of hydrogen was consumed. The warm solution was filtered, and the filtrate was evaporated to dryness *in vacuo*. Recrystallization of the residue from water yielded 11.5 g. (74% yield) of a white solid which decomposed rapidly at 299° with evolution of gas. The infrared absorption spectra of the products prepared by both methods were identical.

Anal. Calcd. for $C_5H_{16}N_8 \cdot H_2SO_4$: N, 39.2. Found: N, 39.2.

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Pyrimidines. IV. 2-, 5-, and 2,5-Substituted Chloropyrimidines

HERMAN GERSHON,[1] RICHARD BRAUN, ALFRED SCALA, AND RAYMOND RODIN

Pfister Chemical Works, Inc., Ridgefield, New Jersey

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In our studies on ring-polychlorinated pyrimidines, it became desirable to prepare a number of analogs with substituents in the 2-, 5-, and 2,5-positions.

The preparation of the 2-substituted 4,6-pyrimidinediols and the corresponding dichloropyrimidines (CH_3,[2] C_2H_5,[3,4] C_3H_7,[5] and C_6H_5[6]) has been reported. Of the corresponding 4,5,6-trichloropyrimidines, only the 2-methyl and 2-chloromethyl analogs are known.[7] In the 5-substituted barbituric acid and 2,4,6-trichloropyrimidine series, the methyl,[8] bromomethyl,[7,9] ethyl,[10] *sec*-butyl,[11] and phenyl[12,13] derivatives are also known. 5-Propyl- and 5-isopropylbarbituric acids had also been reported.[14]

In this study ten additional ring-polychlorinated pyrimidines and the necessary intermediates will be described. Scheme I indicates the synthetic sequence employed.

The appropriate amidine or urea was condensed with the corresponding ethyl malonate in the presence of sodium ethoxide to form a 4,6-pyrimidinediol (I), which was then treated with phosphorus oxychloride, phosphorus oxychloride–dimethylaniline, or phosphorus oxychloride–pentachloride to yield II. Compounds of IIi and IIj were converted to the 5-bromo-

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