tained *via* the sulfonyl chlorides or from the reaction of 2,4-dinitrobenzenesulfenyl chloride, were: 4-chlorophenyl, 168–169°; 4-bromophenyl, 189–190°; 4-*t*-butylphenyl, 128–129°; 2-thienyl, 142–143°; and 4-biphenylyl, 169–170°. These melting points are in agreement with the values obtained by Bost, Turner and Norton[9] and Bost, Turner and Conn.[10] In no case was the melting point of a sulfone obtained from a sulfonyl chloride lowered by the addition of the sulfone with the same molecular formula obtained using 2,4-dinitrobenzenesulfenyl chloride. Biphenylyl 2,4-dinitrophenyl sulfone is yellow; the other sulfones reported above are nearly colorless. 4-*t*-Butylphenyl 2′,4′-dinitrophenyl sulfone, which is apparently new, was also obtained by oxidation of the derivative from *t*-butylbenzene with chromic acid.

Anal. Calcd. for $C_{16}H_{16}N_2O_6S$: C, 52.74; H, 4.43. Found: C, 52.76; H, 4.68.

Acknowledgment.—The authors wish to express appreciation to the Abbott Laboratories of North Chicago, Illinois, for a fellowship grant in support of this work. The microanalyses for carbon and hydrogen were carried out by the late Dr. Gertrud Oppenheimer and by G. Swinehart of the California Institute of Technology.

Summary

2,4-Dinitrobenzenesulfenyl chloride has been found to undergo a Friedel–Crafts type reaction with a variety of aromatic compounds.

The 2,4-dinitrophenylthio group has been shown to enter the 4-position of several monoalkyl- and monohalobenzenes, the 1-position of naphthalene, and the 2-position of thiophene.

The reaction offers an excellent method for the characterization of aromatic compounds.

LOS ANGELES, CALIF. RECEIVED FEBRUARY 13, 1950

[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL]

Pteridine Studies. I. Mercaptopteridines

BY EMERY M. GAL[1]

The observations on tumor-growth influencing properties of folic acid analogs and some pteridines[2] led us to undertake the synthesis and biological assay of a series of known and of hitherto unrecorded pteridines. The results obtained with 2,4-diaminopteridine,[3,4] and with 4-aminopteroylglutamic acid[5] led to a more extensive study of the 4-amino substituted pteridine ring. It was of interest to note how the mercapto group in the 2 position influenced this activity.

Synthesis of 2-mercapto-4,6,7-trihydroxypyrimido(4,5-b)pyrazine was reported by Elion and Hitchings,[6] 4-hydroxy-2-mercaptopyrimido(4,5-b)pyrazine by Polonovski, Vieillefosse and Pesson[7] and the 4-amino-6,7-dihydroxy-2-mercaptopyrimido(4,5-b)pyrazine by Wieland and Liebig.[8] These syntheses were repeated and confirmed.

The method of synthesis is based on the condensation of 2-mercapto-4,5,6-triaminopyrimidine and of the 4,5-diamino-6-hydroxy-2-mercaptopyrimidine with dicarbonyl reagents such as diacetyl, glyoxal, pyruvic acid and oxalic acid. The condensation with pyruvic acid led to the formation of isomers.[6] This was now confirmed with mer-

(1) U. S. Public Health Special Fellow. This work was supported by a grant from the Cancer Research Grants Branch, U. S. Public Health Service, to D. M. Greenberg.

(2) (a) Lewissohn, Leuchtenberger and Leuchtenberger, *Proc. Soc. Exp. Biol. and Med.*, **56**, 144 (1944); (b) Thiersch and Phillips, *Am. J. Med. Sci.*, **217**, 575 (1949).

(3) LaDu, Fineberg, Gal and Greenberg, *Proc. Soc. Exper. Biol. and Med.*, in press (also Abst. 115th National Meeting of A. C. S., San Francisco, March, 1949).

(4) Thiersch and Stock, *Cancer*, **2**, 863 (1949).

(5) Olson, Hutchings and SubbaRow, *J. Biol. Chem.*, **175**, 359 (1948).

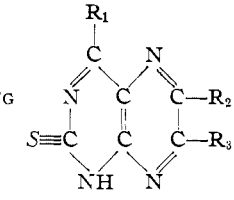
(6) Elion and Hitchings, THIS JOURNAL, **26**, 2553 (1947).

(7) Polonovski, Viellefosse and Pesson, *Bull. soc. chim.*, [5] **12**, 78 (1945).

(8) Wieland and Liebig, *Ann.*, **555**, 150 (1944).

TABLE I

THE 2-MERCAPTOPTERIDINE RING



Compound	R_1	R_2	R_3	U. V. spectra in 0.1 *N* NaOH Max. Å. min. *p*H 8.55–9.0		Soly. in phosphate buffer at *p*H 7.4 mg./100 ml.
				3870	3300	
I	NH_2	..	..	2930	2650	5
				3150 shoulder		
II	NH_2	OH	OH	2700	2460	125 (8)
				3570	3100	
III	NH_2	CH_3	OH	2675	2525	25
				3545	3325	
IV	NH_2	OH	CH_3	3025	2790	110
				3920	3495	
V	NH_2	CH_3	CH_3	3115	2890	3
				3750	3300	
VI	OH	..	..	2870	2620	250 (7)
				3180	2800	
VII	OH	OH	OH	2520	2320	125 (6
				3525	3325	
VIII	OH	OH	CH_3	3020	2700	80
				2515	2400	
				3420	3325	
IX	OH	CH_3	OH	3015	2750	120
				2545	2425	
				3850	3350	
X	OH	CH_3	CH_3	3020	2690	45
Pyrimidines:						
(a) 4,5-Diamino-6-hydroxy-2-mercapto				2725	2455	
(b) 2-Mercapto-4,5,6-triamino				2640	2580	
				2350 shoulder		

captopteridines also through their different absorption spectra and solubilities.

The pteridines studied are listed in Table I. Their ultraviolet absorption spectra in 0.1 *N* NaOH at 10^{-6} mole concentration were determined with a recording spectrophotometer. Higher molar concentrations strongly absorb in the visible spectrum range as all of them display a yellowish tint, particularly between *p*H 8.0–12.0. None of the mercaptopteridines show fluorescence in the ultraviolet when tested with an ultraviolet lamp (G. E. BH4). They are, however, oxidizable with hydrogen peroxide into their intensely fluorescing 2-hydroxy derivatives as recorded by Polonovski.[7] It was observed that mercaptopteridines in quantities of 5–10 γ, while tested for their R_f values by paper chromatography on Whatman No. 1 filter paper strips in 70% collidine (collidine/water) became fluorescent without hydrogen peroxide treatment after forty-eight hours of air-drying. This fluorescence was independent of whether the paper was previously irradiated with ultraviolet or not. This effect was due to a photochemical oxidation, as mercaptopteridines in 70% collidine exposed to ultraviolet for a period of twenty-five minutes without any further treatment showed fluorescence. This, however, was not duplicated if the pteridines were run in butanol–acetic acid–water mixture, which implied increase of ease of oxidation with increasing *p*H.

The mercaptopteridines in contrast to some of the aminopteridines[9] lend themselves to microanalysis without difficulty.

Experimental

4-Amino-2-mercaptopyrimido(4,5-b)pyrazine (I).—Ten g. (0.042 mole) of 2-mercapto-4,5,6-triaminopyrimidine bisulfite[9,10] was dissolved in 150 ml. of water containing 15 g. (0.068 mole) of glyoxal bisulfite, and refluxed for two hours. After the first twenty minutes of refluxing 15 ml. of glacial acetic acid was added. On cooling the crude orange-yellow crystals were filtered, washed with water and dried. The crystals weighed 6.5 g. (86.6%).

For elementary analysis and subsequent biological tests the crystals were dissolved in a minimal amount of 2 *N* NaOH and boiled with charcoal. The solution was filtered and carefully adjusted with glacial acetic acid to *p*H 3.0. After cooling yellow crystals which separated were collected, washed thoroughly with successive portions of water, alcohol and ether and dried at 100° *in vacuo*. The fine yellow needles darkened around 270° but did not melt or decompose around 300°.

Anal. Calcd. for $C_6H_5N_5S$ (179.0): C, 40.03; H, 2.89; N, 39.3; S, 17.9. Found: C, 40.02; H, 2.97; N, 38.5; S, 17.0.

4-Amino-7-hydroxy-6-methyl-2-mercaptopyrimido(4,5-b)pyrazine (III).—Two grams of 2-mercapto-4,5,6-triaminopyrimidine bisulfite and 2 g. of pyruvic acid were boiled in 250 ml. of 0.1 *N* acetic acid for two hours, then cooled and left to crystallize overnight. The crystals were collected and dissolved in 0.5 *N* NaOH. Charcoal was added and the solution was boiled for five minutes. It was filtered while hot and the substance was precipitated at *p*H 3.5 with glacial acetic acid. This was repeated three times and then the fine small yellow crystals were collected, washed with water, alcohol and ether and dried. The crystals melted at 225° with decomposition; yield, 1.2 g. (68.1%).

Anal. Calcd. for $C_7H_7ON_5S$ (209.0): C, 40.19; H, 3.30; N, 33.01; S, 15.26. Found: C, 39.86; H, 3.49; N, 32.82; S, 15.47.

4-Amino-6-hydroxy-7-methyl-2-mercaptopyrimido-(4,5-b)pyrazine (IV).—Two g. of 2-mercapto-4,5,6-triaminopyrimidine bisulfite and 2 g. of pyruvic acid were refluxed in 250 ml. of 2 *N* sulfuric acid for two hours and then left to crystallize overnight. The compound was purified from 0.5 *N* NaOH. The charcoal treated hot alkaline solution was adjusted to neutrality with 2 *N* hydrochloric acid and left to crystallization.[6] At a close neutrality only sulfur separated out which was removed by centrifugation. The supernatant solution was adjusted to *p*H 3 with concentrated hydrochloric acid and left to crystallization. The crystals were dried at 100° under vacuum. The crystals sintered at 217° but did not melt below 300°; yield, 1.04 g. (60.0%).

Anal. Calcd. for $C_7H_7ON_5S$ (209.0): C, 40.19; H, 3.30; N, 33.01; S, 15.26. Found: C, 40.02; H, 3.52; N, 33.10; S, 14.98.

4-Amino-6,7-dimethyl-2-mercaptopyrimido(4,5-b)pyrazine (V).—Two g. of 2-thio-4,5,6-triaminopyrimidine, 2 g. of biacetyl and 2 g. of acetic acid in 100 ml. of water were refluxed for two hours. After cooling overnight the deep yellowish crystals were collected and dissolved in 1 *N* NaOH. The solution was boiled and treated with charcoal. It was then filtered into 1 *N* acetic acid solution and left to crystallization. The fine yellowish crystals charred around 280° but did not decompose at 300°. The yield was 1.04 g. (40.0%).

Anal. Calcd. for $C_8H_9SN_5$ (207.0): C, 46.37; H, 4.34; N, 33.81; S, 15.45. Found: C, 46.17; H, 4.39; N, 33.25; S, 13.60.

4,6-Dihydroxy-7-methyl-2-mercaptopyrimido(4,5-b)pyrazine (VIII).—Three g. of 4,5 diamino-6-hydroxy-2-mercaptopyrimidine[11] and 3 g. of pyruvic acid were boiled in 150 ml. of 2 *N* sulfuric acid for two hours, then cooled and filtered. The filtrate was refluxed for another hour, then concentrated under vacuum to half of its volume. The solution was then put in the ice-box and left to crystallization. The yellow crystals were collected, dissolved in 0.1 *N* NaOH, boiled, treated with charcoal and filtered into 1 *N* acetic acid. After a day of standing the crystals were collected, dried and analyzed; 1.63 g. (42%). The crystals did not melt or decompose between 290–300°.

Anal. Calcd. for $C_7H_6O_2N_4S$ (210.0): C, 40.00; H, 2.87; N, 26.75; S, 15.25. Found: C, 40.61; H, 2.52; N, 26.30; S, 14.96.

4,7-Dihydroxy-6-methyl-2-mercaptopyrimido(4,5-b)pyrazine (IX).—This compound was prepared according to the method given for compound VIII, with the difference that the condensation took place in 2 *N* acetic acid. The yellow crystals sintered at 295° but did not melt at 300°; 2.12 g. (55%).

Anal. Calcd. for $C_7H_6O_2N_4S$ (210.0): C, 40.00; H, 2.87; N, 26.75; S, 15.25. Found: C, 40.23; H, 2.52; N, 26.14; S, 15.05.

6,7-Dimethyl-4-hydroxy-2-mercaptopyrimido(4,5-b)-pyrazine (X).—2.5 g. of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine and 2.5 g. of biacetyl in 100 ml. of 0.1 *N* sulfuric acid were refluxed for three hours. After long standing and cooling to 0°, the yellowish crystals that formed were filtered washed with water and alcohol. The dry crystals were dissolved in hot 0.1 *N* NaOH submitted to charcoal treatment and then filtrate was poured into 1 *N* sulfuric acid. After cooling the separated crystals were collected, washed several times with cold water, cold alcohol and ether, and then dried *in vacuo;* decompose around 280–285°; 2.17 g. (65%).

Anal. Calcd. for $C_8H_8ON_4S$ (208.0): C, 46.19; H,

(9) Mallette, Taylor and Cain, THIS JOURNAL, **69**, 1814 (1947).

(10) Traube, *Ber.*, **33**, 1371 (1900).

(11) Traube, *ibid.*, **37**, 4516 (1904).

3.84; N, 26.90; S, 15.36. Found: C, 46.09; H, 3.94; N, 25.73; S, 14.81.

Summary

Synthesis of several mercaptopteridines in good yields has been described. The ultraviolet absorption spectra of their alkaline solutions and their solubility in phosphate buffer solution has been measured.

BERKELEY, CALIFORNIA RECEIVED NOVEMBER 30, 1949

[CONTRIBUTION FROM THE JOHN HARRISON LABORATORY OF THE UNIVERSITY OF PENNSYLVANIA]

Syntheses in the Oxindole Series[1]

BY E. C. HORNING AND M. W. RUTENBERG[2]

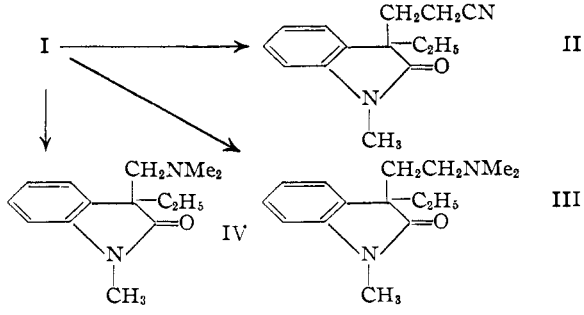
Consideration of the structures of morphine derivatives and synthetic analgesics (Demerol and Amidone series) reveals certain structural features which are common to all; the most important of these appear to be a quaternary carbon atom attached to an aromatic ring) with an amino nitrogen in a beta relationship to it. This might be considered the "essential" structural feature for this type of physiological activity.

In connection with the general problem of the relationship between chemical structure and physiological activity of morphine-type compounds, it has been suggested that compounds containing the "essential" structural features with a nitrogen atom in a position corresponding to the bridge oxygen of morphine would lead to similar physiological effects. It has been postulated[3] that drug molecules adhere to cell surfaces at the site of action by means of specific chemical groups. The possible attachment points of the morphine molecule may be at the basic amino nitrogen and at the oxygen atom of the furan ring (which may form an attachment by hydrogen bonding). A nitrogen atom, replacing the oxygen, would also have free electrons available for hydrogen bonding. Studies of carbazole derivatives, in which nitrogen is in a position corresponding to the bridge oxygen of morphine, have been reported.[4,5] Although these compounds did not show a high order of activity, it was noted that a substituent (methyl, ethyl or acetyl) on the bridge nitrogen led to increased effectiveness with respect to analgesic activity. Oxindoles have been prepared in connection with the synthesis of physostigmine,[6,7] but no record of analgesic activity has been found.

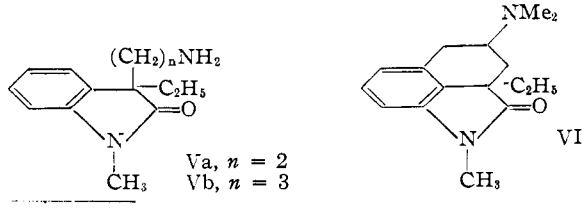
1-Methyl-3-ethyloxindole (I) was prepared by the Stolle method.[8] By employing the Bruson[9] cyanoethylation procedure, the cyanoethyloxindole (II) was prepared. Hydrolysis of the nitrile yielded the corresponding acid, from which a formamide was obtained by the Curtius procedure. This compound was alkylated with a mixture of formic acid and formalin[10] to yield the desired dimethylaminoethyloxindole (III). Alkylation of the oxindole with dimethylaminoethyl chloride gave the same product, in more direct fashion.

For purposes of obtaining information about structure and activity in this series, a group of related amines was prepared. These compounds were designed to test the effect of variation in chain length between the quaternary carbon atom and the amino nitrogen, as well as the effect of substitution on the amino nitrogen.

1-Methyl-3-ethyl-3-dimethylaminomethyloxindole (IV) was prepared by the Mannich reaction. Primary amines of the general structure V were prepared by reduction of appropriate nitriles. In general, reduction with palladium–carbon and platinum catalysts gave mixtures of primary and secondary amines. The next higher homolog of III, 1-methyl-3-ethyl-3-(γ-dimethylaminopropyl)-oxindole



was prepared by methylation of the corresponding primary amine with formic acid and formalin. A



(1) From the dissertation of M. W. Rutenberg, presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy, April, 1949.

(2) Bristol Laboratories Fellow, 1947–1949.

(3) C. C. Pfeifer, *Science*, **107**, 94 (1948); *The Modern Hospital*, **71**, no. 6, 88 (Dec., 1948).

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(9) Bruson and Riener, *ibid.*, **65**, 23 (1943).

(10) E. C. Wagner and E. Staple, *J. Org. Chem.*, **14**, 559 (1949).