$tyr_{1.1}glu_{1.1}his_{0.5}phe_{1.0}arg_{0.9}gly_{1.0}$ϵ-tosyls$_{1.0}$. Met, val and pro present but not determined.

The final product and a number of intermediates were assayed for melanocyte expanding ability,[13,14] with the following results which are expressed in MSH units per gram: his.phe.arg.try.gly 1.5×10^4; his.phe.arg.try.gly.-ϵ-toslys.-pro.val. amide 0.5×10^6; cbzoser.tyr.ser.met.-gluta.his.phe.arg.try.gly.-ϵ-toslys.pro.val. amide 0.8×10^8.

From these results it is apparent that our blocked tridecapeptideamide possesses essentially the same MSH activity as the corticotropins, and that it is one per cent. as active as α-MSH.

(13) K. Shizume, A. B. Lerner and T. B. Fitzpatrick, *Endocrinol.*, **54**, 553 (1954).

(14) We wish to express our gratitude to Drs. A. B. Lerner and M. R. Wright of the Department of Medicine, Yale University School of Medicine, for these assays.

KLAUS HOFMANN
BIOCHEMISTRY DEPARTMENT — MIRIAM E. WOOLNER
UNIVERSITY OF PITTSBURGH — HARUAKI YAJIMA
SCHOOL OF MEDICINE — GERTRUDE SPÜHLER
PITTSBURGH, PENNSYLVANIA — THOMAS A. THOMPSON
ELEANORE T. SCHWARTZ

RECEIVED OCTOBER 10, 1958

REACTION OF BIS-CYCLOPENTADIENYLCOBALT(II) WITH ORGANIC HALIDES

Sir:

Wilkinson[1] has reported that bis-cyclopentadienylcobalt(II) reacts with ethyl bromide to form bis-cyclopentadienylcobalt(III) bromide. We have observed that with certain halogenated hydrocarbons, new covalent cyclopentadienylcobalt compounds are formed as well as the bis-cyclopentadienylcobalt(III) halides. The formation of these new compounds may involve a rearrangement from π bonded cyclopentadienyl rings to σ bonded rings.

We wish to report some preliminary work on the reaction of bis-cyclopentadienylcobalt(II) with carbon tetrachloride.

On the addition of bis-cyclopentadienylcobalt(II) to carbon tetrachloride in an inert atmosphere, bis-cyclopentadienylcobalt(III) chloride precipitates leaving a cyclopentadienylcobalt compound in solution. Removal of the carbon tetrachloride by vacuum distillation leaves a residue which after sublimation and fractional crystallization from hexane has the composition $(C_5H_5)_2CoCCl_3$. Calcd. for $(C_5H_5)_2CoCCl_3$: C, 42.96; H, 3.28; Co, 19.17; Cl, 34.59. Found: C, 42.94; H, 3.46; Co, 19.34; Cl, 33.04. Molecular weight calcd.: 307.5. Found: 315. The yields of the trichloromethyl compound and the *bis*-cyclopentadienylcobalt(III) chloride are 90% and 100%, respectively, based on the equation

$$2(C_5H_5)_2Co + CCl_4 \longrightarrow (C_5H_5)_2CoCCl_3 + (C_5H_5)_2CoCl$$

The trichloromethyl compound, m.p. 79–80°, is orange to red depending on crystal size, decomposes over a period of hours above 40°, and is soluble in organic solvents and insoluble in water. In ethanol–water solutions it reacts slowly to form the bis-cyclopentadienylcobalt(III) cation in greater than 70% yield.

(1) G. Wilkinson, F. A. Cotton and J. M. Birmingham, *J. Inorg. and Nuclear Chem.*, **2**, 95 (1955).

The infrared spectrum of the trichloromethyl compound shows two C–H stretching frequencies, at 3.25 μ and 3.45 μ. Similar frequencies are observed in compounds containing a cyclopentadienyl ring σ bonded to a metal.[2] Cyclopentadienyl rings that are π bonded are known to have only one C–H stretching frequency, in the region 3.20–3.25 μ.[3] The weak absorption at 6.2 μ characteristic of σ bonded cyclopentadienyl rings is also present.

The infrared spectrum indicates that at least one ring in the trichloromethyl compound is σ bonded to the cobalt. In this case, the formation of the compound involves a rearrangement from π to σ bonding. Other indications for σ bonding are that the compound reacts with maleic anhydride in warm benzene and also reacts with ferrous chloride in tetrahydrofuran to form small amounts (about one per cent. yield) of bis-cyclopentadienyliron(II). The other products of these reactions were not identified. Piper and Wilkinson[2] have shown that these two reactions can be used as qualitative tests for a σ bonded cyclopentadienyl group.

Assuming σ bonding for one ring, the reaction with ethanol-water involves a second rearrangement, from σ bonding back to π bonding. Herwig and Zeiss[4] have postulated a similar rearrangement involving $(C_6H_5)_3Cr$. The possibility that the trichloromethyl group is bonded to one of the rings has been considered but discarded as incompatible with this reaction.

Another possible explanation is the formation of a σ bond between the trichloromethyl group and the cobalt, the rings retaining their π character, but sufficiently distorted by the large σ bonded group to account for the infrared spectrum and the chemical reactivity. This would eliminate the necessity for π to σ and σ to π rearrangements.

Further work is at present being carried out to establish the structure and bonding in this compound and in the products of similar reactions with other organic halides. A detailed account of this work will be published in the near future.

(2) T. S. Piper and G. Wilkinson, *ibid.*, **3**, 104 (1956).

(3) T. S. Piper, F. A. Cotton and G. Wilkinson, *ibid.*, **1**, 165 (1955).

(4) W. Herwig and H. H. Zeiss, THIS JOURNAL, **79**, 6561 (1957).

CONTRIBUTION NO. 672 FROM THE INSTITUTE FOR ATOMIC RESEARCH AND DEPARTMENT OF CHEMISTRY
AMES LABORATORY — SEYMOUR KATZ
IOWA STATE COLLEGE — JAMES F. WEIHER
AMES, IOWA — ADOLF F. VOIGT

RECEIVED SEPTEMBER 26, 1958

5-BIS-(2-CHLOROETHYL)-AMINOURACIL, A NEW ANTITUMOR AGENT

Sir:

Since the discovery of the pharmacological properties of methyl-bis-(2-chloroethyl)-amine, HN2[1] and its therapeutic use in human malignancies,[2] a number of analogs and related alkylating agents have been developed which have clinical value.[3] Nevertheless, the need for compounds

(1) A. Gilman and F. S. Philips, *Science*, **103**, 409 (1946).

(2) L. S. Goodman, M. M. Wintrobe, W. Domeshek, M. J. Goodman, A. Gilman and M. J. McLennon, *J. Am. Med. Assoc.*, **132**, 126 (1946).

(3) S. Farber, R. Toch, E. M. Sears and D. Pinkel, in "Advances in Cancer Research," J. P. Greenstein and A. Haddow, editors, Academic Press, Inc., New York, N. Y., Vol. IV, 1956, pp. 20–33.

exhibiting greater oncotoxicity and less general toxicity than those now available still remains. Our interest in replacing the methyl group of HN2 with physiologically active carrier groups led to the synthesis of 5-bis-(2-chloroethyl)-aminouracil, which has been found to be a particularly active carcinostatic and carcinolytic agent in animals.

5-Bis-(2-chloroethyl)-aminouracil (I) was prepared by treating 5-aminouracil with ethylene oxide in aqueous acetic acid to give 5-bis-(2-hydroxyethyl)-aminouracil (II), which was then chlorinated with thionyl chloride in diethylene glycol dimethyl ether in the presence of traces of water and ethanol.

I melts at 206° (dec.); $\lambda_{max.}$ (0.01 N H_2SO_4 in 95% ethanol) 257 mμ a_M 5,675; found C, 48.54; H, 4.44; N, 16.67; Cl, 27.81. II melts at 166–168°; $\lambda_{max.}$ (0.01 N H_2SO_4 in 95% ethanol) 258 mμ—a_M 5,600; 204 mμ—a_M 8,250; found C, 44.50; H, 6.55; N, 19.2.

I has been shown to have an LD_{50} (acute) in rats and mice of about 3.7 mg. per kg. intraperitoneally and about 7.5 mg. per kg. orally. It has minimal hepatotoxicity and shows no automatic or cardiovascular activity in anesthetized dogs. It is not a uracil antagonist for *E. coli* Bu^- which requires this metabolite.

When 5-bis-(2-chloroethyl)-aminouracil is given at the maximum tolerated dose every four to seven days to rats bearing established tumors it is highly effective in causing regression of the tumors without the manifestation of permanent toxicity. The preferred method of dosing is to give the drug orally at 2 mg. per kg. every four days or at 4 mg. per kg. initially and then after seven days at 2 mg. per kg. every fourth day as required. Using these methods the drug is active as a carcinolytic agent against the Walker 256 carcinoma, Jensen sarcoma,

TABLE I

	Days after tumor implantation	Size of tumors mm.	Regressions
Tumor control	13	19	..
	20	39	..
	45	79	..
5-Bis-(2′-chloroethyl)-aminouracil	13	22	..
	20	17	3
	45	1	7
	50	0	8

Walker 256 carcinoma implanted in male rats. I given in 10% ethanol–90% saline: 4 mg./kg. on 13th day, 2 mg./kg. on 18th and 22nd days. 5/9 control animals dead on 45th day, remainder moribund. 8/9 treated animals alive and free of tumors on 50th day.

and Murphy–Sturm lymphosarcoma when the tumors in each case are 10–20 mm. in diameter and have been growing seven to fourteen days. Table I indicates the type of response found with the Walker tumor and is illustrative of that uniformly obtained in the case of other tumors.

The drug shows a linear dose-response curve with respect to inhibition of early transplants when given daily, but under this method of administration the general toxicity is cumulative and the agent is therefore ineffective in causing regression of established tumors. I has also been shown to cause marked or complete inhibition of a wide spectrum of tumors including Sarcoma 180, Cloudman S91 melanoma, Carcinoma 755, and Leukemia L1210 in mice.

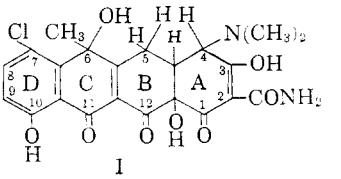
RESEARCH LABORATORIES D. A. LYTTLE
THE UPJOHN COMPANY H. G. PETERING
KALAMAZOO, MICH.

RECEIVED NOVEMBER 7, 1958

THE BIOLOGICAL REDUCTION OF 7-CHLORO-5a(11a)-DEHYDROTETRACYCLINE TO 7-CHLOROTETRACYCLINE BY STREPTOMYCES AUREOFACIENS

Sir:

We have reported recently[1] the characteristics and structure of 7-chloro-5a(11a)-dehydrotetracycline (I), a new tetracycline-like material accumulated by a blocked mutant of *Streptomyces aureofaciens* Duggar, coded as mutant S-1308 and descended from the original 7-chlorotetracycline-producing[2] A-377 soil isolate of Duggar. We also have demonstrated[1] that catalytic hydrogenation of I yields a mixture of approximately equimolar quantities of tetracycline[2] and its epimer, 5a-*epi*-tetracycline.



I

We now wish to describe the biological reduction of I to 7-chlorotetracycline by two *S. aureofaciens* mutants. They are mutant BC-41, a producer of 7-chlorotetracycline, and mutant V-138, a producer of 7-chloro-6-demethyltetracycline[3]; both are descended from the original A-377 soil isolate of Duggar. Addition of I (500 μg./ml.) to 48-hour old fermentation systems, followed by 72 hours of additional fermentation, resulted in reductions by BC-41 and V-138 of 40% and 20% of added I, respectively, the reduction product in both cases being 7-chlorotetracycline. The extents of reduction were estimated by measuring the 7-chlorotetracycline produced; these measurements were carried out by fluorometric assay,[4] by quantitative paper strip chromatography, and by radiochemical methods utilizing 7-chloro^{36}-5a(11a)-dehydrotetracycline. In the radiochemical experiments, no radiochloride ion was observed at the end of the fermentation, the only labeled substances being 7-chlorotetracycline product and unchanged starting material. This observation, taken with the fact that the fermentation contained a large excess of unlabeled chloride, shows that degradation of I to chloride ion, followed by resynthesis to labeled 7-chlorotetracycline, was not occurring. In a similar experiment, BC-41 was grown in the pres-

(1) J. R. D. McCormick, P. A. Miller, J. A. Growich, J. Reichenthal, N. O. Sjolander and A. P. Doerschuk, THIS JOURNAL, **80**, 5572 (1958).

(2) The trademarks of the American Cyanamid Company for 7-chlorotetracycline and tetracycline are Aureomycin and Achromycin, respectively.

(3) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, THIS JOURNAL, **79**, 4561 (1957).

(4) D. H. Feldman, H. S. Kelsey, and J. C. Cavagnol, *Anal. Chem.*, **29**, 1697 (1957).