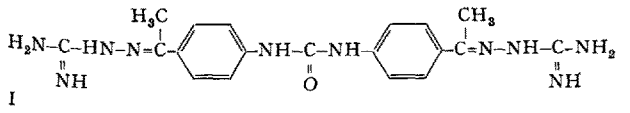
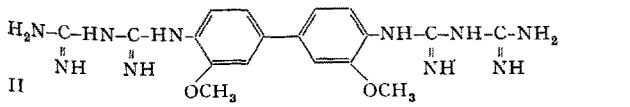
A New *Bis*-Guanylhydrazone with Antileukemic Properties

At the 9th International Cancer Congress MIHICH[1] presented a report on the antileukemic activity of one of our compounds, the *bis*-guanylhydrazone of diacetyldiphenylurea (I), called DDUG. Compound I proved active against leukemias L 1210, P 288, P 388, P 534 JS, and L 5178 Y as well as against lymphoma AK_4.



I

I A 3,3'-analogue of I



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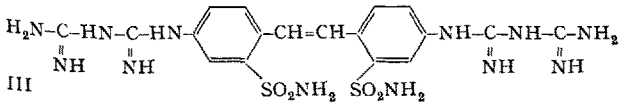

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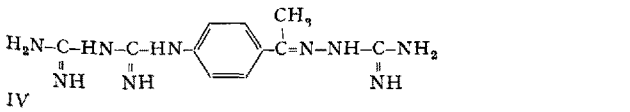

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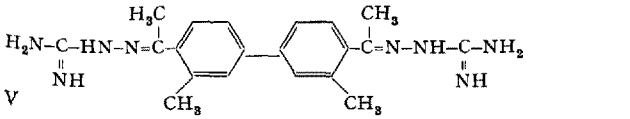

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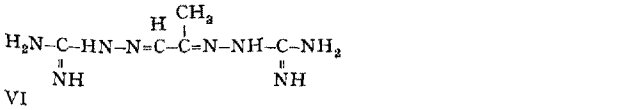


VI

The following is a brief outline of the development of these new guanyl-hydrazones.

The guanylhydrazones, like the biguanides, present a wide variety of interesting pharmacological and chemotherapeutic properties. The monobiguanides, for instance, are of particular interest because of their activity against protozoa[2]. Many years ago, we already found evidence for trypanocidal action in the group of *bis*-biguanides, e.g. in 3,3'-dimethoxy-4,4'-*bis*-biguanido-diphenyl II. In a search for new compounds of similar structure we developed 2 new types, i.e. 2,2'-*bis*-sulphamyl-4,4'-*bis*-biguanido-stilbene III[3] and *p*-biguanido-acetophenone-guanylhydrazone IV[4]. Compounds III and IV displayed excellent activity against various species of *Trypanosoma*, as demonstrated by GOBLE[5]. (According to him IV cures mice infected with *T. rhodesiense*, Wellcome CT-strain and strains 105A and 182A, when given in parenteral doses of 3 · 25 mg/kg. One i.p. dose of 25 mg/kg cured mice with *T. gambiense*, Wellcome TS-strain, III was active against *T. congolense* in mice in doses of 5 mg/kg s.c., in rats in doses of 15 mg/kg s.c. In addition, compound III proved quite effective against *T. cruzi* infection in mice in doses of 12.5 mg/kg given by i.p. injection for 5 days in each of 3 consecutive weeks.) The trypanocidal activity of compound IV was likewise confirmed in trials on human subjects. Compound III was also employed experimentally, with positive results, to treat bovine trypanosomiasis[6]. Of the *bis*-guanylhydrazones synthesized later the 3,3'-dimethyl-4,4'-diacetyl-diphenyl-*bis*-guanylhydrazone – compound V, meanwhile described by EHMER et al.[7] – was inactive against trypanosomes, whereas I exhibited high activity.

MIHICH studied the antileukemic action of a large series of aliphatic *bis*-guanylhydrazones. The compound displaying maximum antileukemic activity was the methylglyoxal-*bis*-guanylhydrazone VI[8] (referred to as CH_3-G). Later we supplied his laboratory with the 3 compounds (I, III, IV) most active against trypanosomes. There was no parallel between the antileukemic activity and the trypanocidal activity: compounds III and IV were inactive against mouse leukemia L 1210, whereas the urea I (chiefly in the form of its *bis*-methanesulphonate) displayed antileukemic activity. Compound V was also inactive in L 1210 mouse leukemia.

For the preparation of compound I, *p*-aminoacetophenone, dissolved in benzene, is treated with phosgene

[1] E. MIHICH, Abstracts of Papers, 9th Int. Cancer Res. Congr., Tokyo 1966.

[2] Cf., for example, chloroguanide (Paludrine®, I.C.I.) CURD, DAVEY and ROSE, Ann. trop. Med. Parasit. *39*, 208 (1945).

[3] A. MARXER, F.P. 1295362; DAS 1217359; USP 3,118,937.

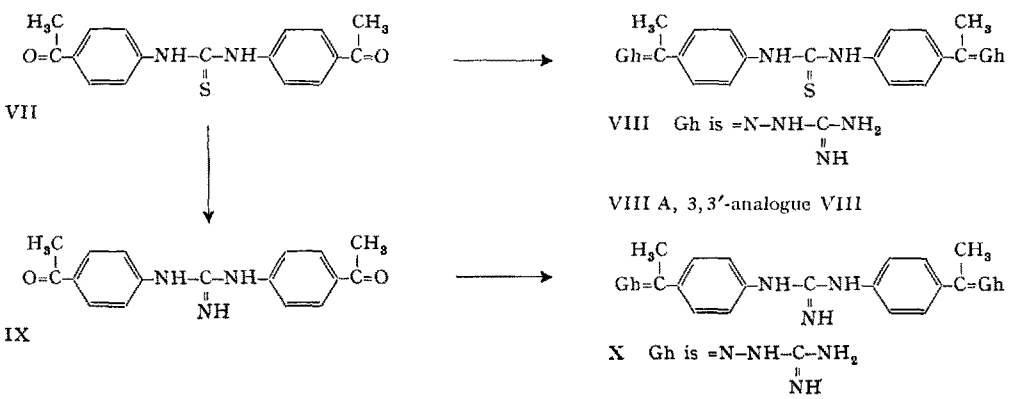
[4] A. MARXER, F.P. 1295366; DAS 1217360; USP 3,211,746.

[5] F. GOBLE, J. Protozool. *9*, Supplement 25 (August 1962) and personal communications. – F. HAWKING, in *Experimental Chemotherapy* (Ed. SCHNITZER and HAWKING; Academic Press, New York 1963), vol. I, p. 205.

[6] W. P. BOYT and H. H. ROTH, Department of Tsetse and Trypanosomiasis, Salisbury (Rhodesia), personal communication.

[7] A. EHMER, K. JAHR, G. KUSHINSKY, K. LÜLLMANN, E. MUTSCHLER and U. WOLLERT, Arzneimittel-Forschung. *14*, 1273 (1964).

[8] E. MIHICH, Cancer Res. *23*, 1357 (1963).



VII

VIII Gh is =N–NH–C–NH_2 (NH)

VIII A, 3,3'-analogue VIII

IX

X Gh is =N–NH–C–NH_2 (NH)

in the presence of triethylamine. The resultant 4,4'-diacetyl-diphenylurea (0.1 *M*) is then made to react with aminoguanidinehydrochloride (0.28 *M*) in a mixture of dimethylformamide and water, containing a slight excess (0.1 *M*) of hydrochloric acid. By adding water, I crystallizes in the form of the di-hydrochloride, which is recrystallized, thus yielding white crystals F.m.p. 238–242° (decomp.). The free base of I is precipitated when a heated solution of the dihydrochloride in water is treated with 2-*n* sodium hydroxide solution. The base I has F.m.p. 222–225° (decomp.). It may be transformed to the *bis*-methanesulphonate by adding methanesulphonic acid to a water-in-alcohol suspension of the base from which the 4,4'-diacetyl-diphenyl-urea-*bis*-guanylhydrazone-*bis*-methanesulphonate I crystallizes on cooling, F.m.p. 247–250°. Hydrochloride and methanesulphonate usually contain 2 *M* of water, but sometimes also 1 or 3 *M*.

The high activity of compound I against leukemia L 1210, prompted the synthesis of a series of analogues. In the same way as above, 3,3'-diacetyl-diphenyl-urea-*bis*-guanylhydrazone (IA) is obtained as a di-hydrochloride-dihydrate which melts at 269–272° (decomp.).

From 4,4'-diacetyl-diphenyl-thiourea (VII), the corresponding *bis*-guanylhydrazone VIII is prepared in a similar way and melts in the form of its dihydrochloride monohydrate at 212–214°.

The corresponding meta-derivative VIIIA, 3,3-diacetyl-diphenyl-thiourea-*bis*-guanylhydrazone-dihydrochloride-monohydrate, was prepared from 3,3-diacetyl-diphenyl-thiourea and had a melting point of 200–205° (decomp.).

By treating VII with one equivalent sodium in alcohol and then with a slight excess of methyliodide, the S-methylderivative is obtained, which – without isolation – is treated with gaseous ammonia yielding 1,3-*bis*-(4-acetylphenyl)-guanidine (IX, F.m.p. 207°). IX was heated with aminoguanidine-hydrochloride, as described above, giving 1,3-*bis*-(4-acetylphenyl)-guanidine-*bis*-guanylhydrazone-trihydrochloride-monohydrate (X) F.m.p. > 310°.

Zusammenfassung. Aus einer Reihe von *Bis*-guanylhydrazonen mit ausgeprägten trypanociden Eigenschaften hat sich eine Verbindung, das 4,4'-Diacetyl-diphenylharnstoff-*bis*-guanylhydrazon I gegen verschiedene Formen von Leukämie (L 1210, P 288, P 534 JS und L 5178 Y) wie auch gegen Lymphoma AK_4 der Maus als wirksam erwiesen. Die Synthese der Verbindung, sowie einiger Analogen wird diskutiert.

A. MARXER

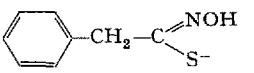
Research Laboratories of the Pharmaceutical Division of CIBA Limited, Basle (Switzerland),
28th November 1966.

The Biosynthesis of the Thioglucoside Moiety of Benzyl Glucosinolate

The biosynthesis of glucosinolates (mustard oil glucosides) has recently been attracting considerable attention. Since it was first shown[1] that the side chains were derived, in many cases, from commonly occurring α-amino acids, several papers have appeared. In particular, it has been demonstrated[2] that the nitrogen and the α-carbon of the α-amino acid are incorporated into the glucosinolate as a unit, and become the nitrogen and carbon of the isothiocyanate group of the aglycone when the glucosinolate is hydrolysed. The efficiency of various sulphur compounds as precursors of both the sulphate and the isothiocyanate sulphur has also been investigated. Sulphur dioxide[3], sulphate[4,5], sulphide, thiosulphate, methionine[5] and cysteine[6] have all been shown to serve as precursors of the 2 glucosinolate sulphur atoms. Methionine was by far the most efficient as a precursor of the isothiocyanate sulphur with an incorporation of sulphur-35 into this position of 9.3%.

We have investigated the incorporation of more complex sulphur-containing compounds labelled with both carbon-14 and sulphur-35 into benzyl glucosinolate (glucotropaeolin) and its aglycone, benzyl isothiocyanate. This glucosinolate is found in relatively large amounts in nasturtiums (*Tropaeolum majus* L.), which were used for this study.

We considered 2 possible alternatives for the addition of the sulphur atom and the glucose group to some nitrogenous derivative of the α-amino acid, viz. either the thiohydroximate aglucone (I) is formed and is glucosylated (the formal reverse of the thioglucosidase cleavage of the glucosinolates), or thioglucose is preformed and introduced as a unit.



I

Sodium phenylacetothiohydroxamate (the sodium salt of I) was synthesized from benzyl chloride by forming sodium dithiophenylacetate ($C_6H_5MgCl + CS_2$) which was subsequently treated with hydroxylamine hydrochloride. Neutralization with ethanolic sodium hydroxide of the hydroxamic acid produced gave the sodium salt. The compound was isotopically labelled by using either benzyl chloride-7-^{14}C or $C^{35}S_2$ as reagents.

Sodium β-D-1-glucopyranosyl mercaptide-^{35}S (sodium thioglucose) was synthesized by treatment of acetobromoglucose with potassium O-ethyl xanthate-^{35}S and subsequent treatment with a solution of sodium in methanol.

The labelled compounds, dissolved in water (100 ml), were administered to young *Tropaeolum majus* plants, freshly cut off just above soil level. The plants were allowed to take up the solution for 72 h, after which they were worked up to isolate the isothiocyanate. The residual solution was assayed for material not absorbed. The plants

[1] M. H. BENN, Chemy Ind. 1907 (1962).
[2] E. W. UNDERHILL and M. D. CHISHOLM, Biochem. biophys. Res. Commun. *14*, 425 (1964).
[3] M. KUTÁČEK, J. SPÁLENÝ and K. OPLIŠTILOVÁ, Experientia *22*, 24 (1966).
[4] H. SCHRAUDOLF and F. BERGMANN, Planta *67*, 75 (1965).
[5] L. R. WETTER, Phytochem. *3*, 57 (1964).
[6] H. KINDL, Mh. Chem. *96*, 527 (1965).