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

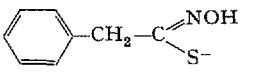
Research Laboratories of the Pharmaceutical Division of CIBA Limited, Basle (Switzerland),
28th November 1966.

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

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were washed free of any adhering radioactivity and macerated in a Waring blender. The homogenate was allowed to stand for 1 h to allow enzymatic hydrolysis of the glucosinolate to take place. A few drops of concentrated hydrochloric acid were then added and the mixture steam-distilled, the distillate being collected in a large excess of aqueous ethanolic ammonia. The distillate was left at room temperature overnight to complete conversion of the isothiocyanate to benzylthiourea (BTU), and then evaporated to dryness. The residue was recrystallized to constant activity from aqueous ethanol. Activities were measured in a liquid scintillation spectrometer.

The results of the feeding experiments (Table I) show that both sodium phenylacetothiohydroxamate and sodium thioglucose appear to be highly efficient precursors,

Table I. Incorporation of sulphur containing compounds into benzylthiourea via the hydrolysis of non-isolated benzyl glucosinolate

Compound administered	Specific activity $\mu c/mM$	BTU specific activity $\mu c/mM$	% incorporation[a]	Dilution ratio[b]
Phenylacetothiohydroxamate-1-^{14}C	9.3	1.53	4.0	6.1
Phenylacetothiohydroxamate-^{35}S	18.8	6.21	4.8	3.0
Sodium thioglucoside-^{35}S	19.1	9.05	12.9	2.1

[a] % incorporation = (activity in BTU/activity absorbed by plant) × 100. [b] Dilution ratio = (specific activity of compound administered/specific activity of BTU).

Table II. Incorporation of phenylacetothiohydroxamate into benzylthiourea via the hydrolysis of crystalline benzyl glucosinolate

Phenylacetothiohydroxamate-^{35}S, specific activity $\mu c/mM$	Benzylthiourea, specific activity $\mu c/mM$	% incorporation	Dilution ratio
23.2	1.09	0.5	21.3

a conclusion that is incompatible with a single mechanism. The apparent incorporation of the phenylacetothiohydroxamate-^{35}S into benzyl glucosinolate was unexpected, and our first supposition was that the thiohydroxamic acid had decomposed to simpler compounds which were then incorporated by the pathway WETTER had demonstrated for sinigrin[5]. However, the carbon-14 labelled thiohydroxamate was also incorporated. Another possibility was that the phenylacetothiohydroxamic acid underwent a Lössen rearrangement in the plant, producing benzyl isothiocyanate which was still present at the time of extraction (or that this decomposition occurred during work-up). This possibility was investigated by feeding the plants with phenylacetothiohydroxamate-^{35}S and isolating the glucoside by the procedure of SCHULTZ and GMELIN[7]. The crystalline benzyl glucosinolate was hydrolysed by incubation with myrosinase to separate the 2 sulphur atoms. The isothiocyanate was isolated by steam distillation as described above. The results are shown in Table II. The low incorporation and high dilution obtained in this experiment strongly indicate that phenylacetothiohydroxamate is not a direct precursor of benzyl glucosinolate.

We therefore conclude that the thioglucoside moiety of benzyl glucosinolate is derived directly from thioglucose. This compound, which is not a common constituent of plants, probably arises by transfer of sulphur from one of the sulphur-containing amino acids to glucose[8].

Zusammenfassung. Phenylacetothiohydroxamat und Thioglukose wurden als mögliche biosynthetische Quellen des thioglukosidischen Schwefelatoms in Benzylglukosinolat (Glucotropaeolin, dem Senfölglukosid der Kapuzinerkresse (*Tropaeolum majus* L.) untersucht. Die experimentellen Ergebnisse deuten darauf hin, dass Thioglukose eine direkte Vorstufe ist, die mit einer von Phenylalanin abgeleiteten Stickstoffverbindung reagiert.

D. MEAKIN

Department of Chemistry, Simon Fraser University, Burnaby 2 (B.C., Canada), 17th November 1966.

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[8] Acknowledgments: Thanks are due to Dr. M. H. BENN for guidance during the early part of this work, and to the National Research Council of Canada for financial support.

Phosphorylation and Uphill Intestinal Transport of Thiamine, in vitro

The hypothesis that thiamine must be phosphorylated by the jejunal epithelial cells for its intestinal absorption, as we suggested in previous papers[1,2], has now been checked by some experiments in vitro, using ^{14}C-labelled thiamine and aspecific metabolic inhibitors or thiamine structural analogues, which specifically affect thiamine phosphorylation. The results we obtained by the everted intestinal sac technique are shortly reported here.

Everted sacs (8 cm long) from the upper small intestine of rats (Wistar strain, 100–150 g body weight) were prepared and incubated for 1 h in Krebs-Henseleit solution[3], as we previously described[1]. The initial concentration of thiamine(thiazole-2-^{14}C) hydrochloride (Radiochemical Centre, Amersham, England; specific activity 0.1 mc/1.26 mg)[4] at both sides of the sacs was 0.2 nM/ml and the

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