After localization of the peaks the corresponding fractions are pooled and the stable iodine determined quantitatively.[3]

With this method it is possible to obtain good separation of the iodothyronines (thyroxine and tri-iodothyronine together in one fraction), iodide, mono- and diiodotyrosine (Fig. 1). The thyronines can then be separated by using, for instance, a suitable paper chromatography method.[1] When about 200 ml of the solvent has passed through the column the eluent is replaced by distilled water. In this way a fifth fraction is obtained which provisionally has been called the non butanol extractable iodine fraction. The total yield has always been above 90 %.

The method described has certain advantages as compared to the paper chromatographic methods that are used for the fractionation of thyroid hydrolysates. The differences will be discussed elsewhere.

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The Structure of Trypacidin

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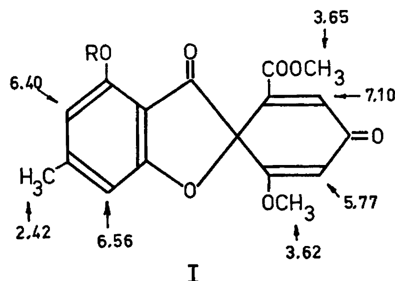
Trypacidin[1] is an antibiotic isolated from *Aspergillus fumigatus*[2] with interesting antiprotozoal properties *in vitro*[2] as well as high activity in experimental toxoplasmosis in mice.[3] Its elementary composition $C_{18}H_{16}O_7$, and a few physical constants have previously been presented.[2] We now wish to report that trypacidin possesses the structure (I, R = CH_3).



I

The ultraviolet and infrared spectra of trypacidin,[2] together with various chemical characteristics, suggested that trypacidin was structurally related to the geodin group of antibiotics. The NMR-spectrum (in CD_3Cl) (Fig. 1) provided an important clue to its detailed structure. Four 3H-singlets at 2.42, 3.62, 3.65, and 3.94 ppm, together with 1H-singlets (broadened) at 6.40 and 6.56 ppm, and 1H-doublets (J = 1.5 cps) at 5.77 and 7.10 ppm, accounted for all sixteen hydrogen atoms. Two protons (6.40 and 6.56 ppm) are clearly positioned *meta* to each other in an aromatic ring substituted with a methyl (2.42 ppm) and a methoxy grouping (3.94 ppm).

* National Science Foundation Postdoctoral Fellow, 1964—1965.

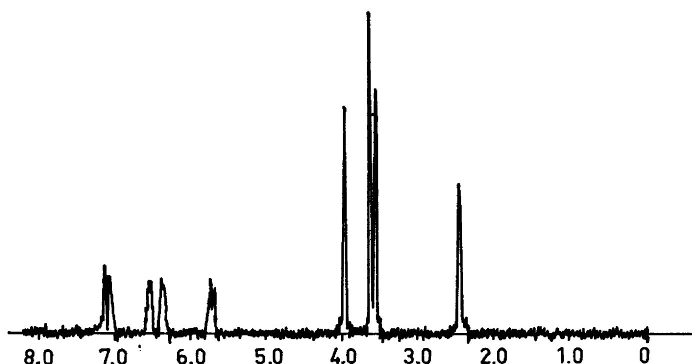


Fig. 1. NMR-spectrum of trypacidin in $CDCl_3$, determined on a Varian instrument at 60 MHz.

The signals at 3.62 ppm and 3.65 ppm can be assigned to a methoxy group positioned at a double bond and a carbomethoxy grouping, respectively, the latter absorbing at 1715 cm^{-1} in the infrared region. Finally, the two low-field olefinic protons (5.77 and 7.10 ppm) can be accomodated in a cross-conjugated dienone system, supported by infrared absorption bands at 1666, 1618, and 1590 cm^{-1}, and by a high extinction band at 287 mμ in the UV-spectrum. The above assignment of NMR-signals is supported by previous studies of griseofulvin derivatives [4] as well as by comparison with an NMR-spectrum of geodin, measured in $(CD_3)_2SO$.* The combined evidence suggested I ($R = CH_3$) as a likely structure for trypacidin.

The corresponding phenol, bisdechlorogeodin (I, R = H), has previously been encountered as a metabolic product produced by various microorganisms. Natori and Nishikawa [5] isolated levorotatory bisdechlorogeodin, among other products, from the mold *Oospora sulphurea-ochracea* v. BEYMA, whereas Stickings and Mahmoodian [6] obtained the dextrorotatory enantiomer from a strain of *Penicillium frequentans*. Finally, Rhodes *et al.*[7] provided evidence for the formation of bisdechlorogeodin, of unstated rotation, in *Aspergillus terreus*. (+)-Bisdechlorogeodin has been converted by Mahmoodian and Stickings [8] into the methyl ether (I, $R = CH_3$) with the reported specific rotations: $[\alpha]_{5790}^{20} + 189 \pm 4°$, $[\alpha]_{5461}^{20} + 221 \pm 4°$ (*c* 1.1, $CHCl_3$).

A homogeneous specimen of trypacidin, produced by preparative thin-layer chromatography (silica gel, benzene:ethyl acetate, 17:3), gave rotation values of $[\alpha]_D^{22} - 160 \pm 4°$, $[\alpha]_{5461}^{22} - 195 \pm 5°$ (*c* 0.36, $CHCl_3$), m.p. 239−240°. A sample of (+)-bisdechlorogeodin methyl ether, kindly provided by Dr. C. E. Stickings, was critically compared with trypacidin with the following results. Determination of the rotation of the (+)-enantiomer in this laboratory gave the values: $[\alpha]_D^{22} + 150 \pm 4°$, $[\alpha]_{5461}^{22} + 177 \pm 4°$ (*c* 0.38, $CHCl_3$). Both compounds had the same melting points when determined in the same bath and exhibited superimposable infrared spectra (in KBr). In addition, trypacidin and (+)-bisdechlorogeodin methyl ether were indistinguishable on thin layer chromatography in two solvent systems, (silica gel, benzene:ethyl acetate, 17:3, 17:8).

We therefore conclude that trypacidin is the levorotatory enantiomer of bisdechlorogeodin methyl ether. The slight deviation in magnitude of rotation may be ascribed to partial racemization of the (+)-enantiomer or both antipodes, taking place during storage or manipulations.

We wish to express our gratitude to Dr. C. E. Stickings, Dept. of Biochemistry, London

* We wish to thank Professor D. H. R. Barton for a generous sample of geodin. Slight deviations were found in the chemical shifts between geodin and trypacidin due to the different solvents employed.

School of Hygiene and Tropical Medicine, (University of London), for providing the authentic reference sample of (+)-bisdechlorogeodin methyl ether.

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Synthesis of *O*-β-D-Xylopyranosyl-L-serine

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In several glycoproteins the sugar and the aminoacid involved in the carbohydrate-protein linkage are *N*-acetyl-D-glucosamine and L-aspartic acid, linked as a 2-acetamido-1-(L-β-aspartamido)-1,2-dideoxy-β-D-glucose residue.

Recent studies, by Rodén and coworkers,[1-3] have indicated that heparin and chondroitin 4-sulphate are linked to protein by a different type of linkage, involving a xylose residue glycosidically linked to the hydroxyl group of L-serine. The synthesis of *O*-β-D-xylopyranosyl-L-serine, which possibly represents the branching point, is therefore a matter of some interest and is reported in the present communication.

Tri-*O*-acetyl-α-D-xylopyranosyl bromide and the methyl ester of *N*-tosyl-L-serine, in a Koenigs-Knorr reaction gave a 70 % yield of the corresponding glycoside. Removal of the protecting groups was expected to offer difficulties, as the electronegative methyl ester group would render the C(3)-*O*-linkage in the serine residue labile to alkali. The ester linkages were hydrolysed by treatment with sodium hydroxide in aqueous methanol and then the *N*-tosyl group was removed by treatment with sodium in liquid ammonia. The yield of the crystalline substance, m.p. 230–240° (decomp.), $[\alpha]_{578} -65°$ (water), was low (1.2 %). On hydrolysis with acid it yielded xylose and serine. It was unaffected by a commercial emulsin preparation which hydrolysed phenyl-β-D-xylopyranoside.

The methyl ester of β-D-glucopyranosyl-L-serine has recently been prepared by Kochetkov and coworkers.[4]

Experimental. Concentrations were carried out under reduced pressure at 40°. Melting points are corrected.

*2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl-*N*-tosyl-L-serine methyl ester.* A mixture of *N*-tosyl-L-serine methyl ester[5] (3.9 g), Drierite (14 g) and freshly prepared silver oxide (3.6 g) in dry, ethanol-free chloroform (14 ml) was stirred in the dark for 1 h. A solution of tri-*O*-acetyl-α-D-xylopyranosyl bromide (5.1 g) and iodine (0.7 g) in chloroform (25 ml) was then added during 1 h and stirring continued for 24 h. After filtration through a layer of Celite, the solution was washed with aqueous sodium thiosulphate and concentrated. Crystallisation from ethanol yielded a product (5.6 g) which gave only one spot on thin layer chromatography (Kieselgel G, ethyl acetate-light petroleum [b.p. 40–60°], 1:1). The crystals melted at 56–60° and on further heating the melt recrystallised at about 100°. When these crystals were used for seeding purposes a product was obtained which melted at 138–139° and had $[\alpha]_{578}^{20} -32°$ (*c* 2.0, chloroform). [Found: C 49.9; H 5.59; O 36.1; N 2.72; S 6.21. $C_{22}H_{29}O_{12}NS$ requires: C 49.8; H 5.50; O 36.1; N 2.64; S 6.02].

O-β-D-Xylopyranosyl-L-serine. The above substance (4.6 g) was dissolved in methanol (20 ml), M aqueous sodium hydroxide (38 ml) was added and the mixture was kept at room temperature under nitrogen for 1 h. Water (50 ml) was added and the solution was