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Quantitative Fractionation of Low-Molecular-Weight Iodine-Containing Compounds in Thyroid Hydrolysates

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There is a need of good and reliable methods for the quantitative fractionation of low-molecular-weight iodinated compounds which at the same time are suitable for automatic recording. The method to be described was developed in this laboratory to meet these demands. The method has already been in use for a long time for the routine fractionation of thyroid hydrolysates and has proved to give reliable results.

A chromatography column about 40 cm × 2 cm in size is packed tightly with a very pure dry cellulose powder (J. H. Munktell's cellulose powder No. 400, Grycksbo Pappersbruk, Grycksbo, Sweden). The cellulose is applied as small discs about 0.5 cm thick. After packing, the column is wetted, starting from the bottom, with the eluent, a butanol-ethanol-ammonia-water solvent system,[1] by a hydrostatic arrangement. The solvent that has passed through is discarded, the direction of flow reverted and the column eluted with 200 ml of the solvent. Before the sample to be analysed is applied on the top of the column, the remaining solvent is sucked off and the column is allowed to drain until the top of the column is almost dry. Good results will be achieved if the volume of the sample does not exceed 0.5 ml. The effluent is collected with a fraction collector. The flow-rate is adjusted with hydrostatic pressure to about 5 ml per hour. A suitable volume for each separate fraction is about 1.5 ml.

If the sample contains radioactive material the radioactivity can be continuously recorded with the aid of a glass spiral passing through a well-type scintillation crystal in the way previously described.[2] Since the flow-rate is fairly constant it is not necessary to use a proportionating pump. The sample can be screened for stable iodine (^{127}I) by the cerium sulphate method,[3] either automatically during the fractionation [4] or from aliquots taken from each separate fraction.

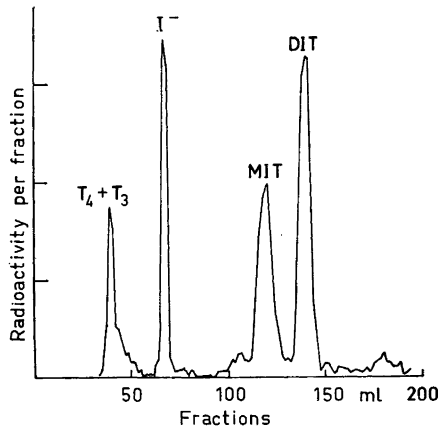

Fig. 1. Fractionation of a hydrolysate from human thyroid tissue. Ordinate: radioactivity in arbitrary units. Abscissa: volume of the eluent (50, 100, 150, and 200 ml).

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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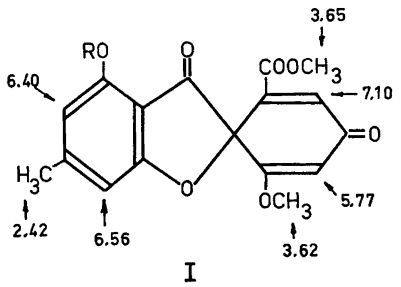
Trypacidin [1] is an antibiotic isolated from *Aspergillus fumigatus* [2] with interesting antiprotozoal properties *in vitro* [2] as well as high activity in experimental toxoplasmosis in mice.[3] Its elementary composition $C_{18}H_{16}O_7$, and a few physical constants have previously been presented.[2] 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).

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