DIGITALIS GLYCOSIDES

THE COLORIMETRIC ASSAY OF THE CHLOROFORM-SOLUBLE GLYCOSIDES OF DIGITALIS

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Received July 2, 1952

ALTHOUGH there are various reports on the assay of tinctures and of pure glycosides of digitalis, the literature contains little information on the evaluation of mixed glycosides. A number of such products are available commercially. We have assayed, biologically and chemically, samples of mixed chloroform-soluble glycosides as being typical of such preparations and also representing intermediate stages in the isolation of digitoxin. We used, for the chemical assay, the colorimetric procedure based on the Baljet reaction,[1] and we have investigated the relationship between this and the biological assay.

EXPERIMENTAL

The chloroform-soluble glycosides were obtained by extraction of the leaf with ethanol, followed by concentration, clarification with lead subacetate and extraction with chloroform. The concentrated chloroform solution was precipitated with light petroleum. Samples described as "digitoxin" are those which give a strong brown junction by the Keller-Kiliani test and which when chromatographed[2] were found to consist mainly of digitoxin. The results of chemical assays by the sodium picrate method and of biological assays, and the ratio of chemical assay to biological potency are shown in Table I, the results being arranged in order of increasing biological assay. The figures for 2 samples of *D. lanata* glycosides are included.

The colorimetric assays were carried out with a reagent consisting of a fresh mixture of 95 ml. of 1 per cent. trinitrophenol solution in water and 5 ml. of 10 per cent. sodium hydroxide solution. 5 to 10 mg. of the sample, according to the expected potency, accurately weighed, was dissolved in 25 ml. of ethanol (70 per cent.). 5-ml. portions were mixed with 5 ml. of reagent and the optical density measured in the E.E.L. Portable Colorimeter after standing for exactly 20 minutes at 20° C. The general purpose green filter No. 404, which has its maximum transmission at about 530 mμ, was used. We did not make use of the lower wavelength suggested in the recent literature[3,4,5,6] because many of our results were already available when these papers were published. The instrument was calibrated in terms of units of activity with digitoxin of known biological potency (950 I.U./g.).

The biological assays were carried out by Dr. H. O. J. Collier using intravenous infusion, from a burette, of a saline preparation of the glycoside. In each assay 5 or more guinea-pigs were used and the standard errors of the determinations were not greater than 8 per cent.

Discussion

With mixed chloroform-soluble glycosides there is no simple relationship between the chemical and biological assays; the chemical assay may be as much as 3 times that of the biological. As the potency increases, with increased digitoxin content, this ratio falls (Table I) and for digitoxin of B.P.C. potency the ratio is close to unity.

It would be desirable to be able to predict the biological potency from the chemical assay but we have found that this cannot be done with any accuracy. Knowing the biological potency, however, it is

TABLE I

Sample	Description	Chemical assay I.U./g.	Biological assay I.U./g.	Assay Ratio chemical/ biological
1	Chloroform-soluble glycosides	467	168	2·78
2	,,	505	197	2·56
3	,,	780	273	2·86
4	,,	746	280	2·68
5	,,	478	304	1·57
6	,,	550	305	1·82
7	,,	765	358	2·14
8	,,	721	358	2·01
9	,,	555	386	1·44
10	,,	690	483	1·43
11	,,	790	526	1·50
12	,,	863	562	1·53
13	"Digitoxin"	955	679	1·40
14	,,	927	681*	1·36
15	,,	982	683	1·44
16	,,	940	690	1·36
17	,,	966	693	1·40
18	,,	890	711	1·25
19	,,	960	760	1·26
20	,,	1026	768*	1·34
21	,,	1050	788	1·33
22	,,	1000	805	1·24
23	,,	908	924*	0·97
24	,,	1074	1073*	1·00
25	,,	1022	1298*	0·78
26	*D. lanata* glycosides	700	493	1·42
27	,,	520	684	0·76

* These figures were obtained by infusion from two joined burettes, one containing an ethanolic solution of the glycoside and the other containing saline solution.

possible to estimate the expected chemical activity with more certainty. It is unfortunate that these estimates are more accurate in the less useful direction, i.e., from biological to chemical. The chemical assay is most useful when considered in conjunction with information as to the origin, colour, solubility, physical constants and chromatographic behaviour of the sample.

We have confirmed that when the aglycones are assayed the colour intensities are twice those produced by equal weights of their respective glycosides. It will be recalled that the molecular weights of digitoxigenin and gitoxigenin are approximately half those of the corresponding glycosides. Thus the presence of aglycone in a mixture will increase the optical density. Conversely, any primary (initial) glycosides will assay low by the colour reaction. Sample 27, for example, was found by chromatography to contain a high proportion of the primary glycosides and in this case the colorimetric assay is lower than the biological. Thus the degree of complexity of the glycoside mixture, namely the relative

proportions of primary and secondary glycosides and aglycones, has an important influence on the chemical assay. The presence of physiologically inactive material giving the Baljet reaction will obviously favour a high chemical assay. With the isolation of digitoxin in mind, the lowering of the chemical/biological ratio will indicate increasing purity of the sample.

SUMMARY

1. It has been shown experimentally that when mixtures of chloroform-soluble glycosides of digitalis are assayed by the Baljet reaction figures are obtained which may be 3 times those by biological assay.

2. This ratio falls with an increase in potency and for digitoxin of B.P.C. potency the ratio is close to unity.

3. It is not possible from the chemical assay of mixed glycosides to estimate the biological activity with any certainty but the assay is valuable when combined with knowledge of the origin, chromatography and physical constants of the material under examination.

We are indebted to Dr. Collier for carrying out the biological assays. Thanks are also due to the Directors of Allen and Hanburys, Ltd., for granting permission to publish these results.

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