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Preparation of Digilanide-A

By OLE GISVOLD and AHYAN ULUBELEN

A method for the convenient isolation of digilanide-A from *Digitalis mertonensis* is described.

PREVIOUS INVESTIGATIONS (1) on the isolation of acetyl digitoxin from *Digitalis mertonensis* revealed that it was the chief desglucoglycoside. Preliminary studies also revealed that the precursor to acetyl digitoxin was digilanide-A. Because this species of digitalis appeared to contain such minor quantities of other glycosides, it suggested the opportunity to use this plant as a possible convenient source of digilanide-A. The experience previously gained in the isolation of the desglucoglycosides was utilized to develop effective methods for the isolation of digilanide-A. Although alcohol and methanol in high concentrations effectively inhibit the enzyme that cleaves the terminal glucose residue of many of the native cardioactive glycosides, they also extract large amounts of chlorophyll and its degradation products. These pigments introduce problems in the subsequent isolation of the native glycosides. Although concentrations of methanol less than 60% have significant inhibitory activity on the glycosidase, concentrations from 60 to 70% are more effective without undue solubilization of chlorophyll. This is true of both the dried and fresh leaves. The pH of aqueous or low hydroalcoholic extracts of fresh leaves of *D. mertonensis* is about 6, and the glycosidase activity is rapid and complete. It also has been shown (2) that this glycosidase is active at a pH of 1. During the drying of the leaves of the various species of digitalis, minimal glycosidase activity is obtained when the leaves are dried rapidly at about 50°. Even on a small scale, it is doubtful that complete glycosidase activity has been arrested. Larger-scale operations prove considerably less effective.

It will be noted under *Experimental* that in the latter steps of the isolation of digilanide-A anhydrous ether was used to extract this glycoside from its dispersion on filter cell together with other substances. In view of the published solubilities of digilanide-A, one might not expect anhydrous ether to dissolve significant quantities of this glycoside, even though it was dispersed on filter cell. Nevertheless, the rate of dissolution is sufficiently great to use this solvent effectively, especially by continued extraction in a Soxhlet extractor. The low boiling point of ether precludes significant decomposition of the glycoside.

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EXPERIMENTAL

The details of the paper chromatographic techniques used in these studies have been described previously (3). Solvent system II was used most extensively for the development of the paper chromatograms. The ascending technique was satisfactory for rapid preliminary screening studies. Decending techniques gave more effective resolution where such was desired. The Raymond reagent was used most extensively to detect the glycosides. Certain flavones gave a yellow color with this reagent. Some unknown impurities gave a brown spot, and still others gave a pink color. The latter might be the digitenolides (4) that are related to diginin, etc., and are physiologically inactive.

Preparation of Digilanide-A

From Dried D. mertonensis.—The leaves of *D. mertonensis* collected in the fall of 1962 were rapidly dried at 50° with the aid of a fan and subsequently powdered and stored in well-sealed glass containers. In preparing a primary extract in which enzymatic activity was inhibited it was shown that 60 to 70% aqueous methanol proved to be quite suitable. The powdered leaves, 400 Gm., were macerated overnight with 1 L. of 65% methanol. The next day they were packed in a percolator, percolated slowly with 65% methanol, and 1300 ml. of precolate was collected. The methanol concentration was reduced to 33% by means of vacuum distillation and the resultant preparation extracted twice with 125 to 150 ml. each of methylisoamyl

ketone–isopropyl ether (1:3). It is advantageous to follow the above extractions with one extraction using 125 ml. of methylisoamyl ketone which removes some yellow pigments together with any desglucoglycosides that may be present. It was demonstrated that methylisoamyl ketone does not extract significant quantities of the native glycosides from about 33% aqueous methanol solutions. The methanol concentration of the primary extract then was reduced to 15% methanol by means of vacuum distillation. Any foaming that might be encountered can be eliminated readily by the addition of methylisoamyl ketone or *n*-octanol. Digilanide-A then can be extracted from this preparation either in a separator or by the use of a Rinco ball extractor using methylene chloride as the solvent. The methylene chloride and any other solvents that might be present were removed completely. The dark yellow-brown residue was digested with methylene chloride that dissolved all the glycosides plus some pigments. The processing of this product to produce a preparation containing chiefly digilanide-A can be carried out as follows. The methylene chloride solution (150 ml. vol.) was diluted with an equal volume of ether, then extracted with several small portions, 5 ml. each, of a 5% potassium hydroxide solution to remove some of the yellow pigments. A small amount of a saturated solution of sodium sulfate facilitated the ready separation of the aqueous alkaline layer. The separation may take place with the resultant formation of a film on the inside of the separator. It was found best to transfer the methylene chloride–ether solution to a second clean separator. Subsequent alkaline washings may have to be treated in a similar fashion. The alkaline washings were followed by a washing with a saturated solution of sodium sulfate containing 4% monobasic sodium phosphate. In the use of the above depigmentation step, care should be taken that the volume of methylene chloride–ether solution is sufficiently large to prevent the separation of digilanide-A during the alkaline extractions. The alkaline extractions are not so effective as when the concentrations of ether to methylene chloride (1) are 3:1; however, significant amounts of pigments can be removed in this way. The organic solvents were removed in the presence of about 6 Gm. of filter cell and dried at room temperature. This preparation was washed with hexane and benzene (60:40) until no more color was removed. Then it was washed with about 100 ml. of isopropyl ether. Finally, the mixture was extracted continuously in a Soxhlet extractor with anhydrous ether until no more extraction was obtained. During this extraction a solid separated out in the flask; it varied in color from white to various shades of light yellow. Examination by paper chromatography revealed that it was composed chiefly of digilanide-A together with small amounts of a slower moving substance. Concentration of the ether mother liquor to a small 10–15-ml. volume caused the separation of more digilanide-A. The ether mother liquor and also the concentrated isopropyl ether and hexane–benzene washings when chromatographed on paper gave a pink spot in the same position as that obtained with digilanide-A. This may possibly be related to the digitenolide (4) type of glycosides. A faster moving pink spot also was detected.

The digilanide-A described above was purified further by redispersing it on new filter cell and subsequent re-extraction with anhydrous ether. Prior re-extraction with hexane–benzene (60:40) may be desirable. The ether was concentrated to a small 25-ml. vol., and about 25 ml. of methylene chloride was added. A large excess of isopropyl ether—about 125 ml.—then was added. The ether and methylene chloride were removed by distillation and the isopropyl ether volume reduced. During this distillation, the contents of the flask were agitated in the presence of suitable boiling chips from a porcelain evaporating dish. The digilanide-A appeared to crystallize out and could be collected readily in a fritted glass filter.

When the hexane–benzene (60:40) and isopropyl ether eluates were examined paper chromatographically, using solvent system II and the Raymond reagent, the presence of digilanide-A could not be detected. They each contained the pink spots and the brown spot described above.

From Fresh D. mertonensis.—The fresh leaves, 750 Gm., were blended with 1200 ml. of methanol at high speed for a few minutes. An additional 300 ml. of 65% methanol was added, and the blending continued for 5 min. The mixture was heated to 60° for several minutes to produce a mixture that readily could be filtered through a wire screen or ordinary coarse filter paper. The marc was washed with additional quantities of warm (60°) 65% aqueous methanol, and a total primary extract of 1850 ml. was obtained. This primary extract was processed in the same way as that obtained from the dried leaves. Less pigments were encountered, and a better digilanide-A preparation was obtained. At certain times of the year, *i.e.*, early fall, the amounts of yellow pigments were so small that quite a suitable digilanide-A preparation could be obtained by the elimination of the alkaline depigmenting step.

In the case of *D. mertonensis*, it also has been demonstrated clearly that by lowering the methanol concentration of the extraction solvent to 33% together with increasing its pH to 7.5 using ammonia that complete inhibition of the enzyme that cleaves the terminal glucose residue of the native glycosides was obtained. These findings have been extended to other species of digitalis, and these results will be reported in a separate communication.

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