Anal.—Calcd. for $C_{17}H_{21}Cl_2N_5O_2$: C, 51.3; H, 5.3; N, 17.6. Found: C, 51.3; H, 5.5; N, 17.5.

3-Methyl-6-(1'-methyl-2'-{*p*-[N,N-bis(β-chloroethyl)amino]benzal} - hydrazino) - 5 - (phenylazo) uracil, m.p. 204°, was obtained from 3-methyl-6-(1'-methylhydrazino)-5-(phenylazo)uracil (20) in 48% yield.

Anal.—Calcd. for $C_{23}H_{25}Cl_2N_7O_2$: C, 55.0; H, 5.0; N, 19.5. Found: C, 54.8; H, 4.9; N, 19.7.

The following compounds were prepared by essentially the same procedure except that 5 ml., rather than two drops, of concentrated hydrochloric acid was added to the reaction mixture. As a result, the desired product was isolated as its hydrochloride.

4 - {*p* - [N,N - Bis(β - chloroethyl)amino] benzalhydrazono} - 5 - bromo - 2 - methylthiopyrimidine hydrochloride, m.p. 210–213° dec., was prepared from 5 - bromo - 4 - hydrazino - 2 - methylthiopyrimidine in 56% yield.

Anal.—Calcd. for $C_{16}H_{18}BrCl_2N_5S \cdot HCl$: C, 38.5; H, 3.8; N, 14.1. Found: C, 38.7; H, 4.0; N. 14.5.

2,4 - Di - {*p* - [N,N - bis(β - chloroethyl)amino]-benzalhydrazono}pyrimidine dihydrochloride, m.p. 180° dec., was prepared from 2,4-di(hydrazino)-pyrimidine (21) in 40% yield.

Anal.—Calcd. for $C_{26}H_{30}Cl_4N_8 \cdot 2HCl$: C, 46.8; H, 4.8; N, 16.8. Found: C, 46.5; H, 4.7; N, 16.7.

2,4 - Di - {*p* - [N,N - bis(β - chloroethyl)amino]-benzalhydrazono} - 6 - methylpyrimidine hydrochloride, m.p. 190° dec., was prepared from 2,4-di(hydrazino)-6-methylpyrimidine (22) in 67% yield.

Anal.—Calcd. for $C_{27}H_{32}Cl_4N_8 \cdot HCl$: C, 50.1; H, 5.1; N, 17.3. Found: C, 49.7; H, 5.4; N, 17.3.

5 - Bromo - 4,6 - di{*p* - [N,N - bis(β - chloroethyl)-amino]benzalhydrazono}pyrimidine hydrochloride, m.p. 207° dec. was prepared from 5-bromo-4,6-di-(hydrazino)pyrimidine in 79% yield.

Anal.—Calcd. for $C_{26}H_{29}BrCl_4N_8 \cdot HCl$: C, 43.8; H, 4.4; N, 15.8. Found: C, 43.4; H, 4.8; N, 15.8.

SUMMARY

A number of hydrazono derivatives of *p*-[N,N-bis(β-chloroethyl)amino]benzaldehyde have been synthesized. The compounds have been submitted for general screening at the Cancer Chemotherapy National Service Center.

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Isolation of Acetyldigitoxin

By OLE GISVOLD

A method for the convenient isolation of acetyldigitoxin from *Digitalis mertonensis* and *D. siberica* is described. The former yielded acetyldigitoxin in approximately twice the amount of digitoxin that could be isolated from *D. purpurea*.

Previous reports (1) on the nature of the chief desglucoglycosides of *Digitalis mertonensis*[1] yielded somewhat inconclusive results. In these investigations both fresh and dried leaves were used together with a number of different extraction conditions and solvents to prepare the primary extracts. Both acetyldigitoxin and digitoxin, when combined, represented the major desglucoglycosides. In one case it was chiefly digitoxin, in another, it was chiefly acetyldigitoxin. It now has been demonstrated that when a primary extract of fresh leaves of *D. mertonensis* is made with 35 per cent methanol and the subsequent purification steps carried out with care, acetyldigitoxin is the chief desglucoglycoside that can be detected and subsequently isolated in good yield. The leaves must be in a healthy condition and free from damaged or partially damaged leaves. Where any amounts of the

Received March 29, 1962, from University of Minnesota College of Pharmacy, Minneapolis.
Accepted for publication June 6, 1962.
This work was supported in part by Research Project Grant NE-06569 from The National Heart Institute of The United Public Health Service.

[1] See *New Phytologist*, **31**, 225(1932).

latter were present, digitoxin also could be detected. Leaves that had been dried rapidly at 50° in 1958 and 1961 yielded chiefly acetyldigitoxin together with very small amounts of digitoxin. Leaves that had been dried less rapidly in 1958, 1959, and 1960 yielded larger amounts of digitoxin together with acetyldigitoxin. Leaves dried at 70° gave results comparable to those dried rapidly at 50°. Drying at 70° was prompted by the discovery (unpublished data) that when *D. siberica* was dried under these conditions the chief desglucoglycoside was digitoxin, while the chief native glycoside is lanatoside A. In the limited number of conditions used to dry the leaves of *D. mertonensis*, varying amounts of deacetylation occurred. As a continuation of this project other drying conditions shall be studied in order to determine if deacetylation can be completely avoided and also if complete deacetylation can be effected. In the latter case, this would lead to the convenient isolation of digitoxin which occurs in about twice the amount that occurs in *D. purpurea* grown in Minnesota.

The chief objective of this report is to describe the convenient isolation of acetyldigitoxin from *D. mertonensis* and *D. siberica*. These techniques also were applied to the isolation of digitoxin from *D. purpurea* with marked success. Aqueous methanol 35 per cent was chosen to prepare an initial (primary) extract in all cases. The desired desglucoglysides readily could be extracted from this initial extract with methyl isoamyl ketone (MIAK). The latter solvent was chosen also because it removed a minimum amount of undesirable substances, can be recovered in good yield, and is not as hazardous as some organic solvents. The initial extract from dried leaves of *D. mertonensis* that had been stored for several years contained degradation products of chlorophyll that carried over into the MIAK extracts and subsequently proved somewhat troublesome. This was not true with freshly dried leaves. Initial extraction of the dry leaves with benzene[2] did not prove advantageous. Extraction of the initial extract with isopropyl ether readily removed these troublesome pigments together with small amounts of acetyldigitoxin and digitoxin. This step may or may not prove advantageous, depending upon the amount of colored degradation products that might be encountered in the primary extract. In the case of carefully collected fresh leaves or carefully dried leaves that are soon used, the extraction with isopropyl ether is not necessary.

[2] With the hope that the benzene soluble degradation products of chlorophyll could be eliminated.

In an attempt to shorten the isolation procedure, the MIAK extract from the primary extract was extracted with aqueous alkali to remove certain yellow pigments. This modification did not lead to deacetylation of acetyldigitoxin if the aqueous alkaline phase was rapidly separated from the MIAK phase. However, fewer pigments were removed by this technique than when they were removed from a mixture of ether-methylene dichloride (3:1).

EXPERIMENTAL

The details of the paper chromatographic techniques used in these studies have been described previously (2). Both solvent systems I and II were used for the development of the paper chromatograms. The Raymond reagent, Kedde reagent, or Jensen's trichloroacetic acid reagent (3) were used to detect the position of the glycosides on the paper. The Raymond reagent readily detected digitoxin and acetyldigitoxin, however, gitoxin was difficult to detect by this reagent. This no doubt was due to the poor solubility of gitoxin. The Kedde reagent readily detected gitoxin and when equal amounts of digitoxin and gitoxin were spotted on paper and then treated with the Kedde reagent, the color intensities were comparable in both cases.

THE ISOLATION OF ACETYL DIGITOXIN

The leaves of *D. mertonensis* collected in the fall of 1961 were rapidly dried at 50° with aid of a fan. The powdered leaves, 250 Gm., were macerated overnight with 1 L. of 35% methanol. The next day they were packed in a percolator, percolated slowly with 35% methanol, and 1500 ml. of percolate was collected. This initial extract then was extracted with three successive portions of 200 ml. each of methylisoamyl ketone by means of a separatory funnel. Some emulsions were encountered that could be broken with anhydrous sodium sulfate. This was the only step that proved somewhat troublesome. Some of the yellow pigments could be extracted from this MIAK extract with aqueous sodium or potassium hydroxide 5%. The MIAK was removed under vacuum (water pump) at 38 to 45°. The dark-colored amorphous mass was digested with methylene chloride which partially dissolved in this solvent. The insoluble yellow-brown solid was removed either by filtration or by use of a centrifuge at 5000 r.p.m., using polypropylene test tubes. This colored insoluble material contained pigments (flavones) that have been partially characterized and will be the subject of a future report. The methylene chloride solution (40 ml.) was diluted with 120 ml. of ether and subsequently extracted with two 10-ml. portions of 5% aqueous potassium hydroxide in order to remove certain yellow flavones and related pigments. These pigments, together with the aforementioned methylene chloride insoluble pigments, which also are alkali soluble, are usually removed by lead acetate or basic lead acetate. We previously have shown that basic lead acetate can effect some deacetylation of the acetyl glycosides. Also, the

use of these reagents for clarification purposes, pigment removal, *etc.*, sometimes results in manipulative techniques that might better be replaced by simpler techniques that accomplish the same results. After washing once with water, the organic solution then was washed with 10 ml. of 20% of sodium phosphate monobasic and then once again with water. Sodium phosphate monobasic is an excellent way to remove the excess alkali in that it is much superior to acetic acid and permits the ready removal by washing of any excess of the acidifying agent. The removal of any excess acetic acid is most troublesome; for at this stage, if the ether methylene chloride solution is allowed to stand overnight, the separation of small amounts of glycosides takes place. However, the solvent was directly removed by distillation until a concentrate of about 20 ml. was obtained. The concentrate was impregnated on filter cell 4 Gm. and dried. The mixture was placed in a sintered-glass funnel and eluted slowly with 150 ml. of a mixture of 60 parts skellysolve B and 40 parts benzene. This solvent mixture removed some nonphenolic pigments and other resin-like substances that have not been characterized. The mixture then was eluted with 150–200 ml. of anhydrous ether. Upon concentration of the ether eluate to a small volume, acetyldigitoxin crystallized out directly in the distillation flask. The glycoside was collected by means of a sintered-glass filter. The filtrate was diluted with an equal volume of skellysolve B (hexane) and additional amounts of glycoside separated out that was collected by filtration. When the paper was examined chromatographically, the above two fractions contained chiefly acetyldigitoxin. The procedure in subsequent isolations was shortened by adding the skellysolve B directly to the ether concentrate and the total glycosides obtained by one filtration. Concentration of the ether–skellysolve filtrate to remove about half the ether caused the separation of further small quantities of acetyldigitoxin together with some green pigments. The filter cell finally was extracted with methylene dichloride to yield an additional small amount of glycosides which could have been extracted very slowly by additional quantities of ether. Of the solvents tested, ether gave the most satisfactory results and gave an initial glycoside preparation that had a very high glycoside concentration consisting almost entirely of acetyldigitoxin.

Acetyldigitoxin also can be eluted from the filter cell mixture by isopropyl ether, and upon concentration of this eluate the glycosides separated out as when ether was used.

When 250-Gm. samples of dry *D. mertonensis* were assayed by the above described techniques, values of the order of 0.55 Gm. of acetyldigitoxin were obtained, or 2.10 Gm. per Kg.

In the case of dried leaves in which some deacetylation had taken place during the drying, the same approximate weights of glycoside were obtained that were mixtures of digitoxin and acetyldigitoxin.

When fresh leaves of *D. mertonensis* were used, the only variation in the above techniques was the disintegration of the fresh with aqueous methanol of such a concentration that the final extract was 35%. With fresh leaves, as previously stated, one can be assured of very little or no deacetylation.

Although no effort was made to isolate or determine the acetylgitoxin or gitoxin fractions, all evidence obtained to date indicates that very small amounts of digilanide B are present in *D. mertonensis*. Contrast this with the large amounts of gitoxin (from purpurea glycoside B) that occur in *D. purpurea* grown in Minnesota.

The same techniques that were used for the isolation of acetyldigitoxin from *D. mertonensis* were applied to both the fresh and dried leaves of *D. siberica*. The results were essentially the same as those obtained with *D. mertonensis*, however, considerably fewer pigments were encountered, especially the flavone type. Quantitative studies will be carried out at a later date and reported.

The Isolation of Digitoxin from *D. purpurea*.—The isolation of digitoxin from *D. purpurea* was accomplished with ease by the same techniques used to isolate acetyldigitoxin from *D. mertonensis*. The gitoxin separated from the methylene dichloride ether solution both before and after concentration and very little gitoxin was encountered in the final digitoxin preparation.

When 250 Gm. samples of dry *D. purpurea* were assayed by the above described techniques, values of the order of 0.225 Gm. of digitoxin were obtained, or 0.90 Gm. per Kg. This is less than half of that obtainable from *D. mertonensis*.

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ERRATUM

In the paper titled "Rheology of Thixotropic Montmorillonite Dispersions II. Kinetics of Structural Recovery" (1), the legend in the caption for Fig. 1 should read: △, 2 weeks; ●, 6 weeks; ○, 11 weeks; and □, 17 weeks. In addition, the reference to Fig. 3 in line 4, paragraph 5, column two, at page 953 should be a reference to Fig. 1.

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