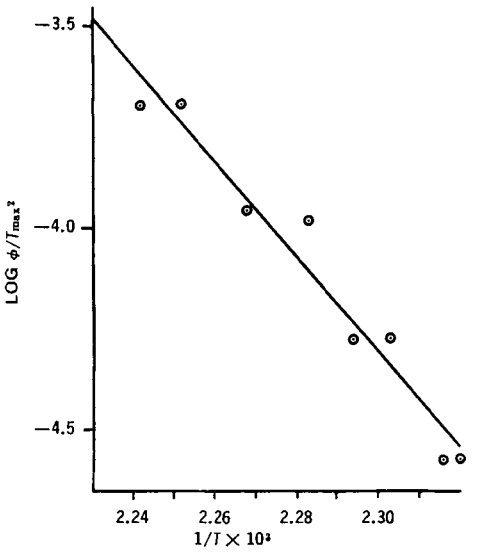

Figure 7—*Plot showing the relationship of heating rate and peak maximum temperature for the transition endotherm in determining the thermodynamic activation energy.*

in which the amount of Form I in the mixtures varied from 20 to 74%. The results of this experiment are shown in Table I. Excellent agreement was found between the experimental and theoretical values. These data show a higher degree of accuracy than was found in the work of Moustafa and Carless (4).

The kinetics of the transformation of sulfathiazole Form I were studied at 395, 415, 420, and 425°K. Figure 2 depicts the change in the transition endotherm as a function of time at 415°K. The fact that the transition endotherm is changing with time indicates that Form I is slowly disappearing. Figures 3 and 4 illustrate the data obtained in the kinetic studies in which a linear relationship resulted when the area of the transition endotherm was plotted *versus* the square root of time. These results suggest that the transition is analogous to the diffusion-controlled crystal growth from solution (5). Reaction rates were determined for the rates of conversion. A typical Arrhenius-type plot of the data is presented in Fig. 5. From these data the apparent heat of activation was determined to be approximately 56 kcal./mole. To verify this, the thermodynamic activation energy for the transition of Form I to Form II was determined by an alternate method (6). Similar to the work of Himuro *et al.* (7), the peak maximum temperature for the transition endotherm was measured at various heating rates. The data are illustrated in Fig. 6. As expected, the peak maximum temperature decreased as the heating rate decreased. Utilizing the following equation of Kissinger (6):

$$\frac{d(\ln \phi/Tm^2)}{d(1/T_m)} = -E/R \qquad \text{(Eq. 1)}$$

where ϕ is the heating rate, and T_m is the peak maximum temperature for transition, a plot of ln ϕ/Tm^2 *versus* $1/T_m$ should yield a straight line with a slope of $-E/R$. This is illustrated in Fig. 7. The energy of activation as determined by this method was approximately 57 kcal./mole. The value is in good agreement with the results obtained isothermally. The data presented in this study suggest that the differential scanning calorimeter can be utilized as a quantitative analytical tool to follow the kinetic transformation of polymorphic materials.

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Acetyldigoxin and Acetyldigitoxin from *Digitalis lanata*

OLE GISVOLD

Abstract □ This paper describes the ready separation of acetyldigoxin from acetyldigitoxin when these secondary glycosides are isolated in combination from *Digitalis lanata*, fresh or dried, containing the maximum amounts of the native glycosides. The initial primary extract used 35% aqueous methanol and enzyme-favoring conditions. Final separation of the two acetyl glycosides was effected by partitioning between 12.5% aqueous methanol and benzene or toluene.

Keyphrases □ Acetyldigoxin—separation from acetyldigitoxin in *Digitalis lanata* extracts □ Acetyldigitoxin—separation from acetyldigoxin in *Digitalis lanata* extracts □ *Digitalis lanata* extracts—separation of acetyldigoxin from acetyldigitoxin

Previous reports described the preparation of acetyldigoxin (1) from *Digitalis lanata* and acetyldigitoxin (2) from *Digitalis siberica* and *Digitalis mertonensis*. In the case of *D. lanata*, an aqueous primary extract of the fresh leaves was used; in the case of *D. siberica* and *D. mertonensis*, 35% aqueous methanol was used to prepare a primary extract from either the fresh or dried leaves. Solubility characteristics of the acetyl glycosides,

together with the nature of the native glycosides and favorable conditions for the latter's enzymatic decomposition, dictated the composition of the solvents used to prepare the primary extracts. It follows that if 35% aqueous methanol were used to prepare a primary extract from fresh *D. lanata* leaves, one would obtain a mixture of the acetyl glycosides, *i.e.*, acetyldigoxin, acetylgitoxin, and acetyldigitoxin. The latter occurs in greater amounts than acetyldigoxin in *D. lanata* and complicated the ready isolation of acetyldigoxin when 35% aqueous methanol was used to prepare a primary extract from the fresh leaves.

Experiences gained in the isolation of acetyldigoxin and acetyldigitoxin from different species of *Digitalis*, as well as the challenge that existed to separate conveniently these two acetyl glycosides when they simultaneously could be obtained together in a primary extract, were the motivating factors for this research project.

Although fresh leaves are preferable for the isolation of the acetyl glycosides, small amounts were available to initiate these investigations. Fortunately, it previously had been established that leaves dried carefully under specified conditions served almost as well as fresh leaves. Dried, powdered *D. lanata*, which contained the native glycosides in large amounts and an active enzyme essential to these studies, was used[1].

The successful separation of a semipurified mixture of acetyldigoxin from acetyldigitoxin is dependent upon the very low solubility of acetyldigoxin in benzene or toluene and the solubility of acetyldigitoxin in these solvents under the conditions of the experiment.

EXPERIMENTAL

The details of the paper chromatographic techniques used in these studies were described previously (3). The Raymond reagent was used to detect the position of the glycosides on the paper. Acetyldigitoxin and digitoxin give a violet color, and acetyldigoxin and digoxin give a blue color.

Isolation and Separation of Acetyldigoxin from Acetyldigitoxin—Carefully dried and finely powdered *D. lanata*, 25 g., was shaken with 200 ml. of 35% aqueous methanol for 1–3 hr. After filtration, the marc was washed with 35% aqueous methanol and a total of 250 ml. was collected. The methanol was removed under vacuum with the bath at 60°. The aqueous preparation, about 160 ml., was extracted with three 50-ml. portions of methyl isobutyl ketone. Some emulsions were encountered that could be broken by a centrifuge at 4000–5000 r.p.m. This step was the only troublesome one due to the emulsions formed. The methyl isobutyl ketone was concentrated to a volume of 20 ml. or completely removed under vacuum with the bath at 60°. At a very low volume of the solvent, crystallization of the glycosides occurred. In the case where all the ketone was completely removed, the solid residue was dissolved in 25 ml. methylene dichloride and then diluted with 75 ml. ether. A clear homogeneous solution was obtained, which was washed with three successive portions of 3 ml. each of 2% potassium hydroxide. The organic layer was transferred to a clean separator and then shaken with 5 ml. of a saturated solution of potassium biphosphate. After filtration the solvent was removed by distillation. In the case where the methyl isobutyl ketone was concentrated to a volume of 20 ml., 60 ml. ether was added and this solution was processed as just described.

The solid residue obtained by either of the described techniques was dissolved in 5–7.5 ml. of methanol. (In the present investigation utilizing the dried powdered *D. lanata*, 6.25 ml. gave the best results.) When 50 ml. of benzene or toluene was added, a clear solution was obtained. In the case where 6.25 ml. of methanol was used, 43.75 ml. of water was added and the mixture was shaken vigorously. Initially, a cloudiness appeared in both the aqueous methanol and the organic phases. The mixture was allowed to stand 1.5–3 hr., after which times some crystallization had taken place in the aqueous methanol layer. Paper chromatographic analysis showed that this crystalline material was acetyldigoxin and that there was some acetyldigitoxin in the aqueous methanol layer.

The organic phase, upon standing several days, yielded almost colorless crystals which gave a single spot on a paper chromatogram with the same R_f value as a reference sample of acetyldigitoxin. More acetyldigitoxin was recovered upon either concentration of the organic solvent *via* distillation or spontaneous evaporation. When a volume of about 5 ml. was reached, dilution while warm with commercial hexane[2] (to obtain a ratio of about 40:60 with benzene or toluene) induced the separation of all but very small amounts of acetyldigitoxin.

The extension of these described techniques to *D. purpurea* led to the preparation of digitoxin, which was found in the benzene or toluene.

It must be pointed out that the maximum amounts of acetyldigoxin and acetyldigitoxin that can be obtained are very dependent upon the nature of the crude drug available. If dried leaves are used, they must be prepared in a way to preserve the greatest percentages of the native glycosides; fresh leaves are preferable to dried leaves. In the limited number of experiments performed by the author, it was noted that the preparation of a very finely powdered leaf *via* the use of a ball mill seemed to lead to some enzymatic decomposition of the leaf as measured by the amounts of digoxin and digitoxin detected. Similar results were observed if dried powdered leaves were allowed to macerate for over 3 hr. Shorter periods led to less amounts of digoxin and digitoxin.

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[1] Supplied by Sandoz Ltd.

[2] Skellysolve B.