Stability of Diosgenin

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The decomposition of 25α-Δ[5]-spirosten-3β-ol (diosgenin) has been studied by various physical techniques in the solid state, in contact with both 2 *N* hydrochloric acid and 2 *N* sodium carbonate solution, and in solution in both chloroform and benzene. In contact with 2 *N* hydrochloric acid, about 40 percent of the diosgenin was found to decompose within 4 months, whereas in the other conditions, the amount of decomposition was found to be negligible, although decomposition products were detected on thin-layer chromatograms and by ultraviolet spectroscopy.

THE STEROIDAL SAPOGENIN, 25α-Δ[5]-spirosten-3β-ol (diosgenin), is of considerable importance as the main precursor of the pharmacologically active steroids, including the corticosteroids and oral contraceptives. Diosgenin is obtained commercially from the tubers of several species of *Dioscorea* by acid-hydrolysis of the naturally occurring saponins. This treatment with acid has been shown to produce products other than diosgenin. Peal (1) showed that when diosgenin was heated under reflux with ethanolic hydrochloric acid, a certain amount of 25α-spirosten-3,5-diene was formed, the amount varying with the acid concentration, being about 35% when refluxed with 2 *N* hydrochloric acid for 2 hr. Blunden, Hardman, and Morrison (2) found that when diosgenin was refluxed with 2 *N* aqueous hydrochloric acid for 2 hr., the quantity of 25α-spirosten-3,5-diene formed was about 6%. Yamagishi (3) found that the optimum procedure for obtaining diosgenin from the saponin dioscin with the least diene formation was refluxing for 2 hr. with 4 *N* sulfuric acid.

After boiling the *Dioscorea* tuber with acid in the extraction procedure for diosgenin, the acid-insoluble material is collected and washed, to free it from acid. Rothrock, Hammes, and McAleer (4) reported that if the residue were not washed free from acid, the diosgenin yield decreased when the residue was dried. Blunden and Hardman (5) found that if the acid-insoluble residue was stored in an acid condition, the diosgenin content decreased. To overcome this problem, Hershberg and Gould (6) neutralized the acid-insoluble material with a base. A similar technique using sodium carbonate solution was used by Blunden and Hardman (5). However, during the commercial extraction procedure of diosgenin, the process is sometimes continued until the acid-insoluble residue is produced this being exported for the final extraction of diosgenin. In certain cases, the shipped material has been distinctly acidic.

As indicated earlier, diosgenin is known to decompose when stored in contact with hydrochloric acid, but the authors are unaware of any published data concerning the stability of this steroid in the solid state or in solution in common organic solvents. The present investigation was undertaken to study the stability of diosgenin under these conditions over a 4-month period and determine the extent of decomposition compared with the total decomposition of diosgenin stored in contact with 2 *N* hydrochloric acid for the same period.

EXPERIMENTAL

The diosgenin used in this investigation was obtained from Steraloids Ltd. After three recrystallizations from acetone the material had a melting point of 204–205°. Decomposition of the sapogenin was studied over a 4-month period in the following environments: as solutions in chloroform and benzene and as solids, which were untreated or in contact with either 2 *N* hydrochloric acid or 2 *N* sodium carbonate solution. Suitable quantities of solutions or solids were packed in sealed glass ampuls at either 4, 20, or 37°, in light or in the dark. The solid samples and solutions in chloroform and benzene were examined at different time intervals, but only one sample of diosgenin in contact with 2 *N* hydrochloric acid and one sample in contact with 2 *N* sodium carbonate solution were prepared, both of which were stored at 20° in the light and examined after storage for 4 months. All the samples were examined by a number of different methods. (*a*) Optical rotations of diosgenin as solutions in chloroform were measured by a Bellingham and Stanley polarimeter using a cell of 1 ml. capacity and 10 cm. path length. (*b*) Infrared spectra of the diosgenin samples were obtained from Nujol mulls, using a Perkin-Elmer 257 spectrophotometer. (*c*) Nuclear magnetic resonance spectra of solutions of diosgenin in chloroform were determined using a Perkin-Elmer R10 spectrometer, utilizing a computer-assisted technique. (*d*) Thin-layer chromatograms were obtained on air-dried Silica Gel G (Merck) layers, 250 μ thick. Two-way chromatograms were prepared using chloroform-acetone (3:1 v/v) as the developing solvent for the first direction and *n*-hexane–ethyl acetate (3:1 v/v) for the second way. Development in each direction was for 15 cm. from the point of application of the test solution. The compounds were

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detected by spraying the plates with a 300% w/v antimony trichloride solution in concentrated hydrochloric acid (7) and heating at 100° for 10 min. (*e*) Ultraviolet spectra of solutions of the sapogenin in chloroform were determined between 250 and 450 mμ using a Unicam SP.800 recording spectrophotometer. (*f*) Melting points were determined on dried material and were uncorrected. (*g*) Quantitative estimations of the diosgenin content of the samples were effected by the densitometric thin-layer chromatographic method of Blunden, Hardman, and Morrison (2). (*h*) Gas-liquid chromatograms were obtained by use of a Perkin-Elmer F11 instrument, stationary phase silicone gum rubber E-301, support A.W.-D.M.C.S. Chromosorb G 80-100 mesh $2^1/_2$: $97^1/_2$. The dual glass columns were maintained at 230°, the injection block temperature was 220°, and the nitrogen flow rate 55 ml. min.$^{-1}$. A standard Perkin-Elmer flame ionization detector was used. Signal amplification was 1×10^2, and sample volume 3 μl. of a 0.4% w/v solution.

RESULTS AND DISCUSSION

Of the techniques used to follow the decomposition of diosgenin, measurement of optical rotation proved of no utility, as no significant change in this property was detected in any sample. This indicates that groups immediately adjacent to the optical center are unaffected by the decomposition process.

The infrared and nuclear magnetic resonance spectra showed marginal changes in some samples, but in general, these techniques seem of little use in examining unresolved decomposition mixtures of diosgenin.

Thin-layer chromatography proved a most useful technique for following decomposition of the steroid. The diosgenin sample that had been stored in contact with 2 *N* hydrochloric acid, when examined by TLC, showed only two antimony trichloride positive spots, one being unchanged diosgenin and the second being a faint spot of high R_f value, which cochromatographed with 25α-spirosten-3,5-diene (Fig. 1). The chromatographic picture differed from that of a sample of diosgenin that had been refluxed with 2 *N* hydrochloric acid for 2 hr., before TLC examination (Fig. 2). These results indicate that the mechanism of decomposition resulting from refluxing diosgenin with acid may be different from that

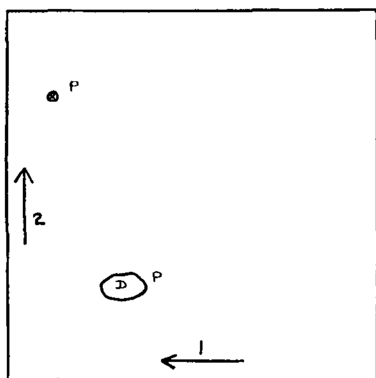

*Fig. 1—TLC of diosgenin stored in contact with 2*N *HCl for 4 months. Solvent system: (1) chloroform–acetone (3:1 v/v; (2)* n-*hexane–ethyl acetate (3:1 v/v). Key: D, diosgenin; X, 25α-spirosten-3,5-diene; colors produced, p, purple.*

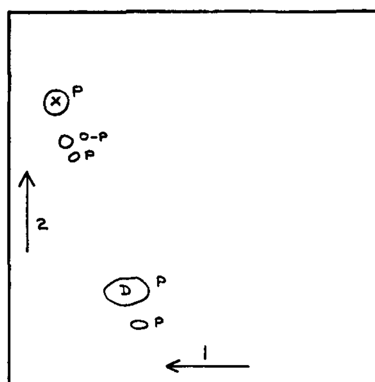

Fig. 2—TLC of diosgenin after 2 hr. reflux with 2 N *HCl. Solvent system: (1) chloroform–acetone (3:1 v/v), (2)* n-*hexane–ethyl acetate (3:1 v/v). Key: D, diosgenin; X, 25α-spirosten-3,5-diene; colors produced, op, orange purple; p, purple.*

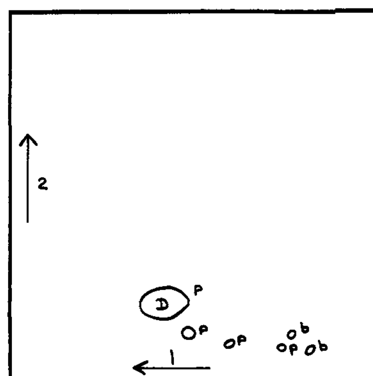

Fig. 3—TLC of diosgenin stored in chloroform solution for 4 months. Solvent system: (1) chloroform–acetone (3:1 v/v), (2) n-*hexane–ethyl acetate (3:1 v/v). Key: D, diosgenin; colors of spots, p, purple; b, blue.*

operating when the compound is stored in acid at 20°. Moreover, these mechanisms appear to be different from that operating in the other test samples, in which no trace of 25α-spirosten-3,5-diene was detected. Thin-layer chromatograms prepared from diosgenin samples stored in the solid state and as solutions in chloroform and benzene were very similar (Fig. 3).

Further evidence of decomposition was obtained from the ultraviolet spectra, absorbance in the region of 250–350 mμ increasing during the storage period; no peaks were detected in the 350–450 mμ region During the decomposition process the absorbance in the 250 mμ and 285 mμ region increased. The ultraviolet spectrum of diosgenin stored in chloroform is markedly different from that of the drug stored in contact with acid or of pure diosgenin (Fig. 4). This is further evidence of differing decomposition routes. The ultraviolet spectrum of diosgenin with decomposition products is similar to that of 25α-spirosten, 3,5,-diene prepared synthetically. However, measurement in the critical 230–250 mμ region was not possible since the chloroform "cut-off" wavelength is about 245 mμ. The diene was not detected by thin-layer chromatography in diosgenin decomposition samples and thus its extent of formation may be presumed to be very low.

The melting point of decomposition samples de-

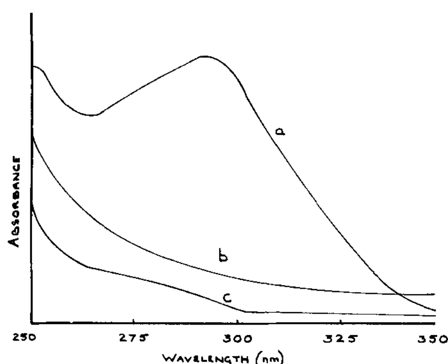

Fig. 4—Ultraviolet spectra of (a) *diosgenin stored in chloroform,* (b) *stored in contact with 2* N *hydrochloric acid, and* (c) *pure diosgenin.*

creased progressively from 204–205° to about 193°, but then remained stationary.

Quantitative TLC studies showed that, at the end of 4 months, the diosgenin had decomposed by an amount that was insignificant in all samples except the one that had been in contact with acid, of which approximately 40% had decomposed (Table I). The quantitative results obtained were subject to an experimental error of ±7% (2). At first sight this result is somewhat surprising, since all samples assayed after 4 months showed several decomposition compounds. However, antimony trichloride solution is a sensitive locating reagent for steroids, able to detect minute quantities of material. From this it was apparent that evaluation of qualitative thin-layer chromatograms of diosgenin for decomposition could be misleading since, even though several decomposition products may be present, the extent of the decomposition may be negligible.

Results obtained from GLC studies also indicated that the extent of decomposition was very low. Thus 4-month-old solid samples of diosgenin, as well as solutions in both chloroform and benzene, showed only two very small signals in addition to the diosgenin peak (Fig. 5). Apparent retention times were diosgenin 29 min. and decomposition peaks 18 and 38 min. Decomposition signals are obviously insignificant in comparison with the unreacted diosgenin.

The effect of temperature and light on the decomposition did not appear important. Qualitatively very similar chromatograms were obtained from all decomposition samples, and no evidence was obtained of photo or thermal effects on the rate of decomposition.

TABLE I—DIOSGENIN DECOMPOSITION AFTER DIFFERENT TIME INTERVALS

Diosgenin Samples	Diosgenin Yield Percent of Original After 1 month	2 months	3 months	4 months
Solution in benzene[a]	98.9	100.5	98.5	99.1
Solution in chloroform[a]	100.3	100.8	98.7	97.6
Solid[a]	99.5	97.8	100.1	98.5
In contact with 2 *N* HCl[b]	...	...	...	60.0
In contact with 2 *N* Na_2CO_3[b]	...	...	...	101.1

[a] Samples stored in the light at 37°. [b] Samples stored in the light at 20°.

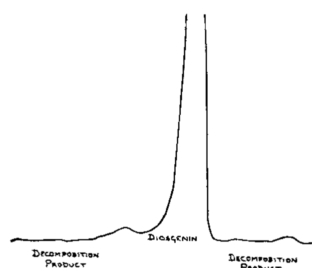

Fig. 5—GLC of diosgenin stored in chloroform for 4 months.

In this study it has been demonstrated that diosgenin rapidly decomposes in contact with hydrochloric acid. During the extraction process of diosgenin from plant sources, it is obvious that after hydrolysis of the naturally occurring saponins to liberate diosgenin, it is imperative to remove the excess acid before processing or transportation of the acid-insoluble residue. The process of neutralizing any residual acid with a base, such as sodium carbonate solution, is a sensible precaution as it does not cause any significant deterioration of diosgenin, even when the two are stored together for 4 months.

When the steroidal constituents of *Dioscorea* species have been studied by paper and TLC, many different spots have been detected. *Dioscorea* species contain both sterols and saponins and as sterols are known to decompose both in the solid state and in solution (8, 9), it will be certain that many of the spots produced on chromatograms of *Dioscorea* extracts will have been produced either during the acid treatment of the plant material to liberate the sapogenins or the subsequent decomposition of the steroidal constituents, in the solid state or in solution (10).

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Keyphrases

Diosgenin stability
Hydrochloric acid—diosgenin solid
Sodium carbonate solution—diosgenin solid
Chloroform—diosgenin solution
Benzene—diosgenin solution
TLC—analysis, decomposition
UV spectrophotometry—analysis, decomposition
GLC—analysis, decomposition