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# Catalog of Infrared Absorption Spectra of Steroidal Sapogenin Acetates

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**A very convenient tool in the identification of a steroid is its infrared spectrum. The infrared absorption spectra between 700 and 1400 cm.$^{-1}$ are presented for the acetates of twelve of the most common sapogenins derived from plant saponins. The spectra are highly characteristic and make possible a ready identification of an unknown sapogenin by comparison of its spectrum with these reference curves.**

THIS laboratory is engaged in a large scale investigation of the steroidal sapogenins that occur in the plant kingdom. Pure sapogenins have been isolated and identified by methods described in a previous paper (*10*). As a means of characterizing these compounds, their infrared absorption spectra have been obtained. The spectra so obtained are highly characteristic of the individual sapogenins and constitute a more positive identification of unknown sapogenins than any other combination of physical constants.

A sapogenin can be identified either by means of its spectrum in chloroform or by means of the spectrum of its acetate in carbon disulfide. Although both of these methods are used routinely in this laboratory, the acetate spectra are found to differ more from one another than do the spectra of the free sapogenins. Hence an unknown can be identified with greater certainty by means of its acetate spectrum, especially if it is not completely pure. In this paper, reference absorption spectra are presented for the most common sapogenin acetates.

## EXPERIMENTAL

Details of the methods of separation and purification of the sapogenins are given in a previous paper (*10*). Table I gives the plant source and physical constants of each sapogenin. The infrared absorption curves were obtained with a Beckman IR-3 spectrophotometer, using sodium chloride prisms. Concentrations were approximately 10 grams per liter, in cells approximately 1 mm. thick.

Since the earlier manuscripts of this series were first written, the wave-length scale of the infrared instrument has been recalibrated. A slight shift from the earlier calibration was found, probably due to a small temperature difference resulting from an improvement in temperature control equipment. Wave numbers reported in this paper and in the first of the series (*10*) are correct. Some of the wave numbers appearing in the second (*9*) and third (*8*) papers of the series are in error by approximately

**Table I. Physical Constants of Sapogenins**

| Compound | Plant Source | Melting Point[a], ° C. | | Specific Rotation[b], Degrees | | Estimated Purity, % |
|---|---|---|---|---|---|---|
| | | Genin | Acetate | Genin | Acetate | |
| Chlorogenin | *Chlorogalum pomeridianum* | 269–273 | 154–155 | −75 | −40 | 98–100 |
| Diosgenin | *Dioscorea composita* | 200 | 196–199 | −121 | −121 | 95–100 |
| Gitogenin | *Yucca filamentosa* | 271–272 | 244–245 | −75 | −103 | 98–100 |
| Hecogenin | *Agave*[c] | 265–268 | 247–250 | +6.6 | −1.2 | 98–100 |
| Kammogenin | *Yucca filamentosa* | 243–244 | 260–263 | −50 | −81 | 98–100 |
| Kryptogenin | *Dioscorea*[d] | 174–177 | 146–150 | −183 | −175 | 90 |
| Manogenin | *Yucca gloriosa* | 245–247 | 248–250 | +2 | −50 | 90 |
| Samogenin | *Yucca carnerosana* | 198–205 | 190–192 | −74 | −84 | 80 |
| Sarsasapogenin | *Yucca cf. thornberi* | 199–202 | 133–141 | −89 | −75 | 90–100 |
| Smilagenin | *Agave lecheguilla* | 180–184 | 150–152 | −75 | −50 | 90–100 |
| Tigogenin | *Yucca gloriosa* | 207–211 | 210–212 | −65 | −72 | 98–100 |
| Yuccagenin | *Yucca sp.* | 239–241 | 178–180 | −96 | −140 | 98–100 |

[a] Kofler melting points.
[b] All rotations at 25° C. in $CHCl_3$, 589 mμ, concentrations of 8 ± 1 grams per liter.
[c] Repurified from mixed batches from different species.
[d] Repurified from a sample obtained from Syntex, S.A., Mexico, D.F.

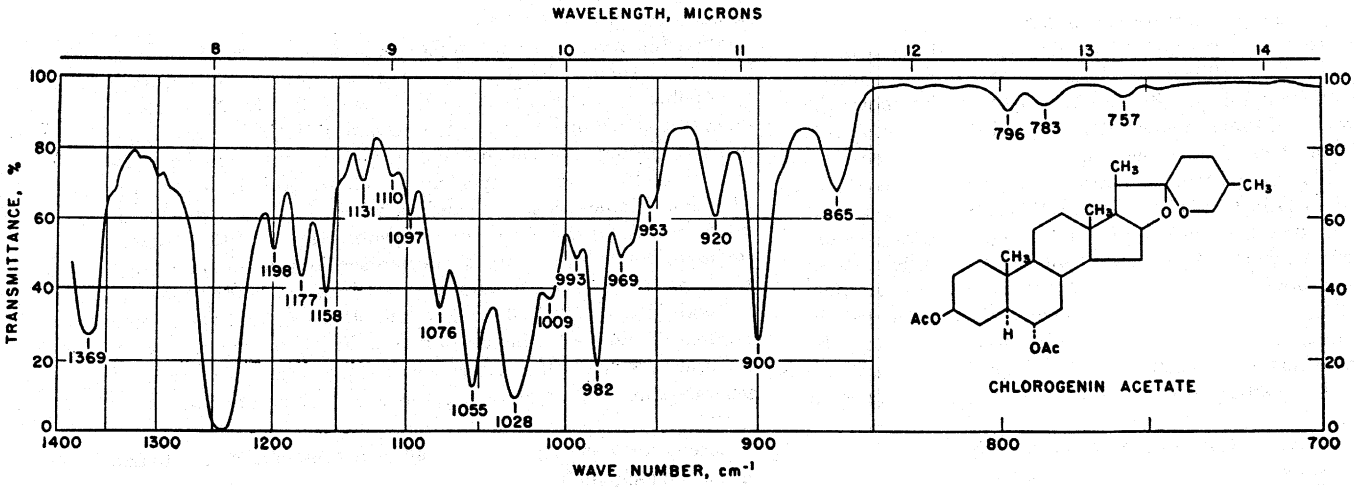


**Figure 1. Chlorogenin Acetate (22-Isoallospirostan-3β,6α-diol 3,6-Diacetate)**

**9.9 grams per liter in $CS_2$. 1.1-mm. cell**

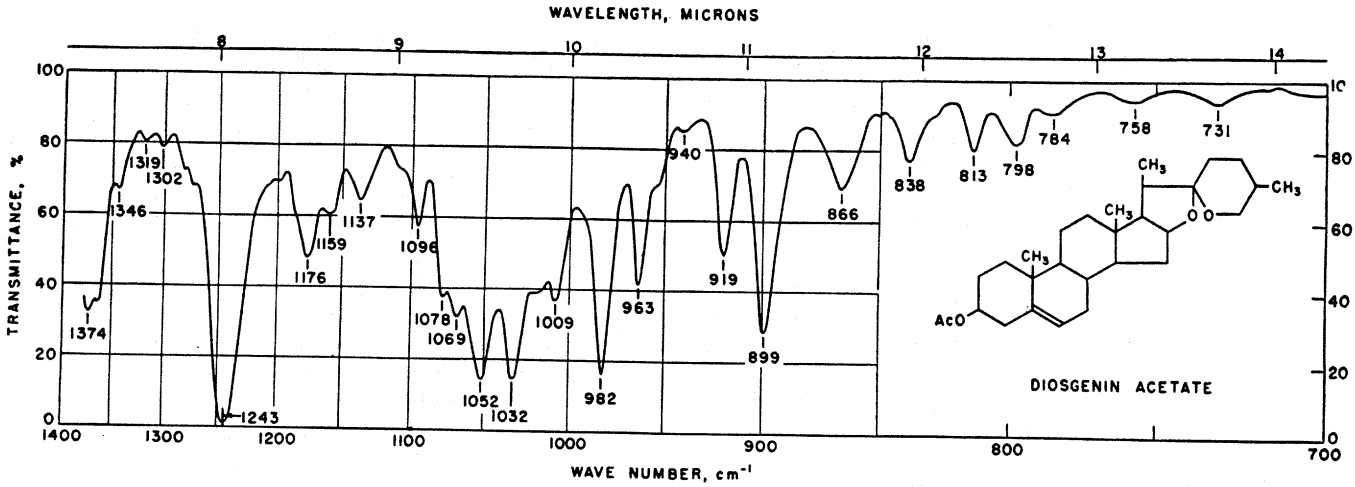


**Figure 2. Diosgenin Acetate ($\Delta^5$-22-Isospirosten-3$\beta$-ol 3-Acetate)**

**10.0 grams per liter in $CS_2$. 1.1-mm. cell**

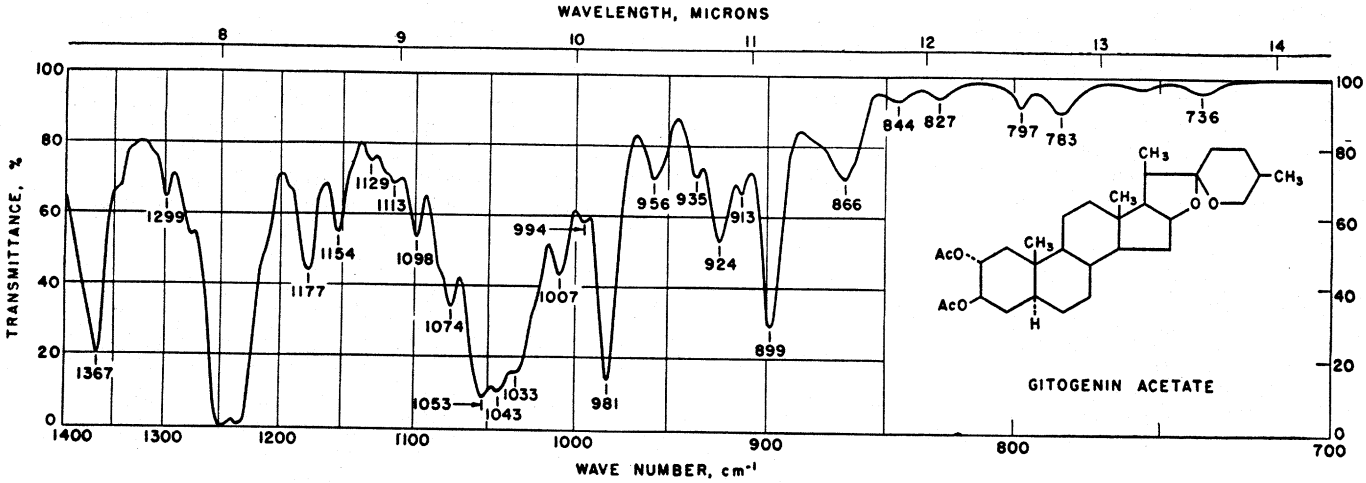


**Figure 3. Gitogenin Acetate (22-Isoallospirostan-2$\alpha$,3$\beta$-diol 2,3-Diacetate)**

**10.0 grams per liter in $CS_2$. 1.1-mm. cell**

1 cm.$^{-1}$; correct values of some of these can be obtained from the current paper or from the first paper.

## DISCUSSION OF SPECTRA

Although the complete spectra from 670 to 5000 cm.$^{-1}$ have been obtained, the portions useful for differentiating sapogenins are the fingerprint region from 700 to 1400 cm.$^{-1}$ and the carbonyl region from 1600 to 1800 cm.$^{-1}$ Figures 1 to 12 give the fingerprint spectra from 700 to 1400 cm.$^{-1}$ Figure 4 also shows the carbonyl region of hecogenin acetate, a typical 12-keto sapogenin. The 1736 cm.$^{-1}$ band is present in all the sapogenin acetates (1735 and 1736 in the monoacetates, stronger in the diacetates and shifted to 1736 to 1744). The 1712 cm.$^{-1}$ band is present only in the keto sapogenins (1714 in kammogenin and manogenin, 1720 in kryptogenin). As curves of all the sapogenin acetates of a given carbonyl class are essentially the same between 1600 and 1800 cm.$^{-1}$, this region of the spectrum is not reproduced in this publication for any except hecogenin acetate. It may be noted that, although the acetate band and the 12-keto band are well resolved in carbon disulfide, they are not well resolved in chloroform.

A previous paper (*9*) mentioned the usefulness of the four absorption bands near 860, 900, 920, and 980 cm.$^{-1}$ for identifying steroidal sapogenins as a class and for differentiating between iso and normal configurations of the *F* ring. It can be seen that all of the spectra presented here possess these four bands, with the exception of kryptogenin, the structure of which lacks the *E* and *F* rings.

The acetate bands in the 1240 cm.$^{-1}$ region are characteristic of the steric relationship between the $C_5$ hydrogen and the $C_3$ acetate, as shown by Jones *et al.* (*4*). Molecules with a cis relationship have a more complex system of bands (Figures 8, 9, and 10) than molecules with either a trans relationship or a 5,6 double bond (Figures 1 to 7, 11, and 12). In the steroidal sapogenins, this makes it possible to determine the type of *A*/*B* ring fusion, since the configuration at $C_3$ is always $\beta$ in the naturally occurring steroidal sapogenins. Thus a complex band near 1240 cm.$^{-1}$ indicates cis ring fusion, whereas a single band indicates trans (allo) ring fusion or $\Delta^5$ unsaturation. With the sapogenins there is also not much interference from a second acetate group, as the 1240 cm.$^{-1}$ band is merely broadened or doubled by a diacetate (Figures 1, 3, 5, 6, 7, and 12) with a shape clearly distinguishable from the greater complexity of the cis bands (Figures 8, 9, and 10). These spectra also agree with the other generalizations of Jones *et al.* for the simpler steroids (*2*, *3*, *5*, *6*).

Except for kryptogenin, these spectra show absorption bands in the regions 1038 to 1056, 1063 to 1098, 1124 to 1143, and 1158 to 1190 cm.$^{-1}$, as found in ketals by Bergmann and Pincha (*1*). However, unlike their open-chain ketals, the 1063 to 1098

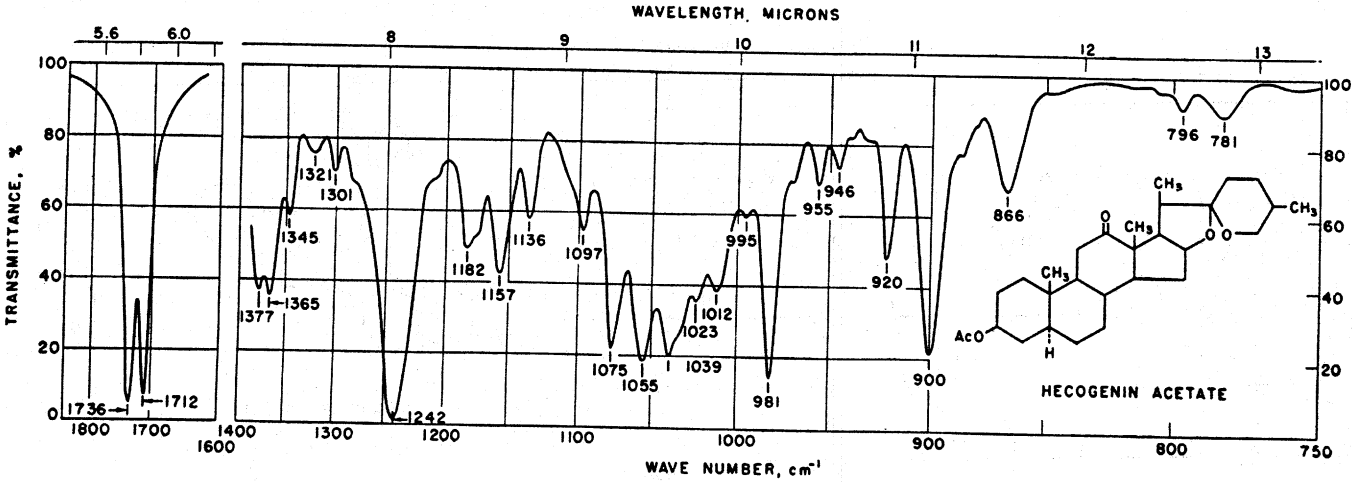


**Figure 4. Hecogenin Acetate (22-Isoallospirostan-3β-ol-12-one 3-Acetate)**

10.0 grams per liter in $CS_2$. 1.1-mm. cell. This sample has no absorption bands between 700 and 750 cm.$^{-1}$

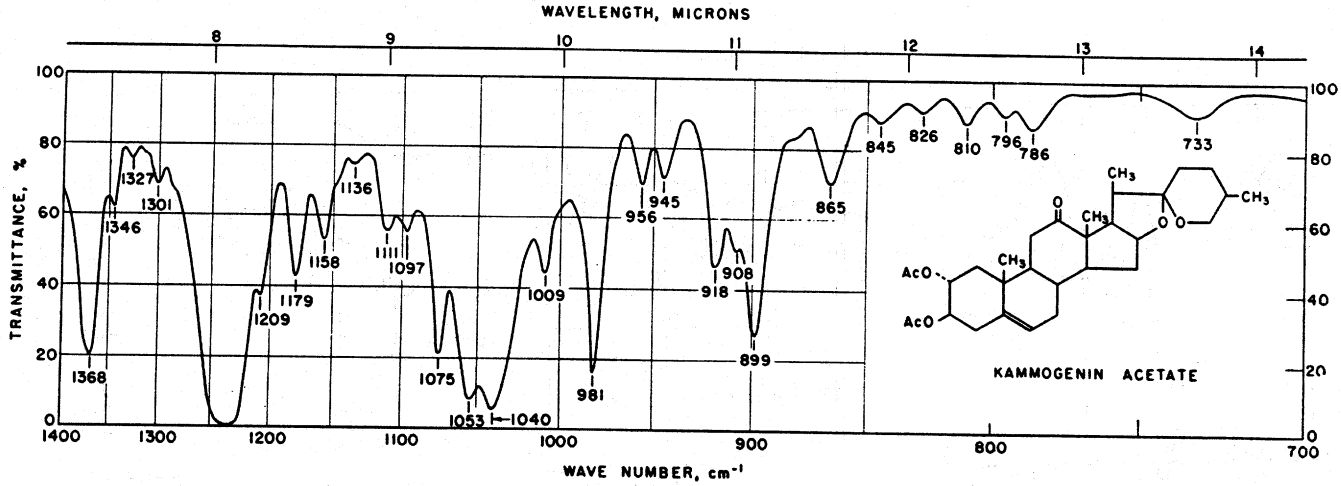


**Figure 5. Kammogenin Acetate ($\Delta^5$-22-Isospirosten-2α,3β-diol-12-one 2,3-Diacetate)**

10.0 grams per liter in $CS_2$. 1.1-mm. cell

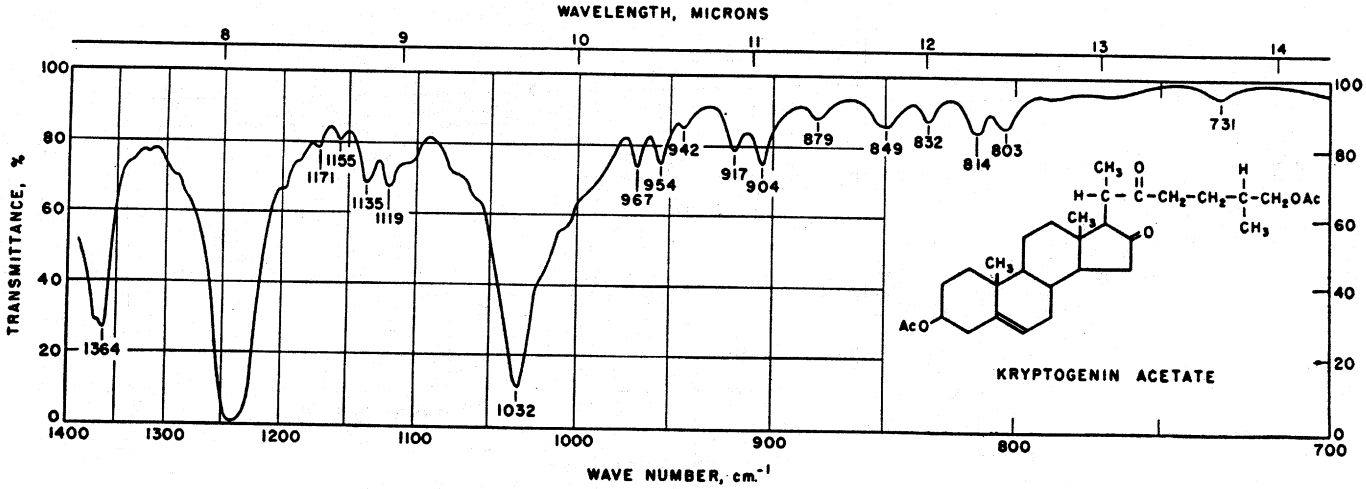


**Figure 6. Kryptogenin Acetate ($\Delta^5$-Cholesten-3β,26-diol-16,22-dione 3,26-Diacetate)**

10.0 grams per liter in $CS_2$. 1.1-mm. cell

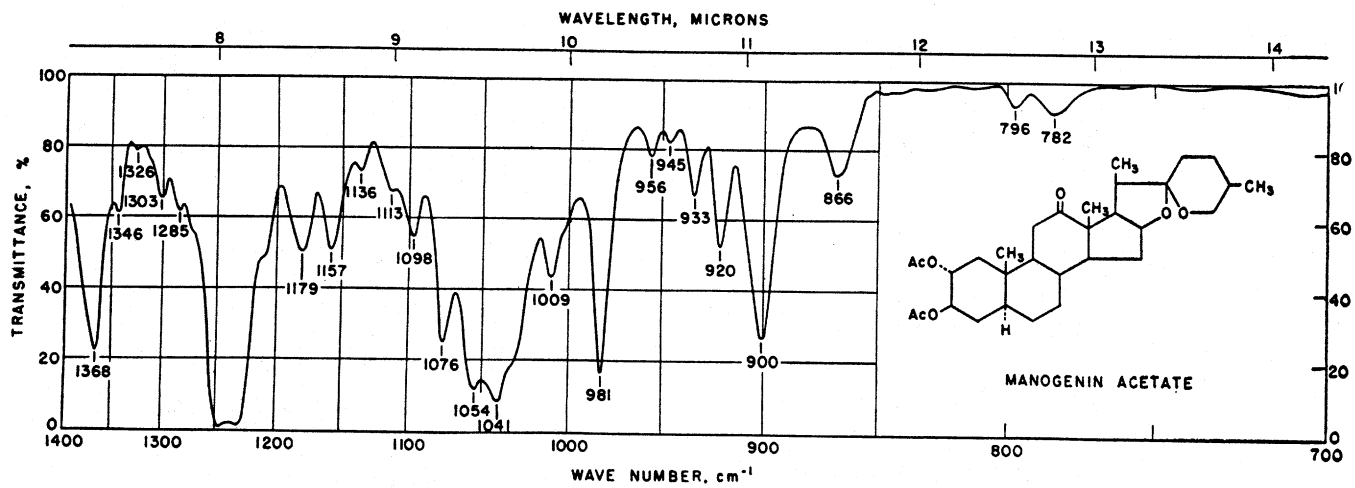


**Figure 7. Manogenin Acetate (22-Isoallospirostan-2α,3β-diol-12-one 2,3-Diacetate)**

9.9 grams per liter in $CS_2$. 1.1-mm. cell

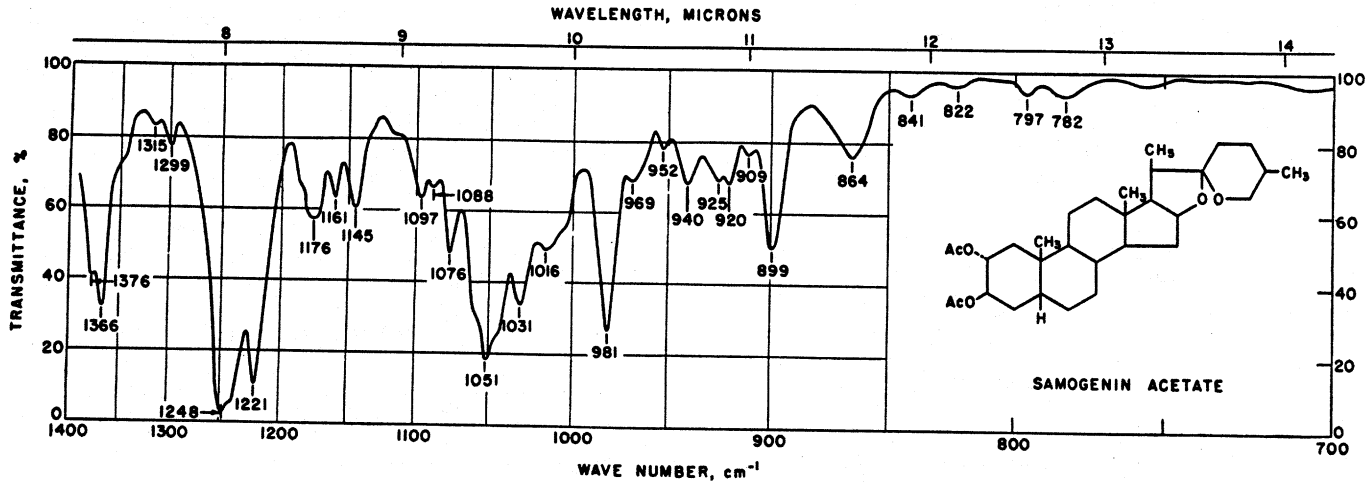


**Figure 8. Samogenin Acetate (22-Isospirostan-2α,3β-diol 2,3-Diacetate)**

7.0 grams per liter in $CS_2$. 1.1-mm. cell

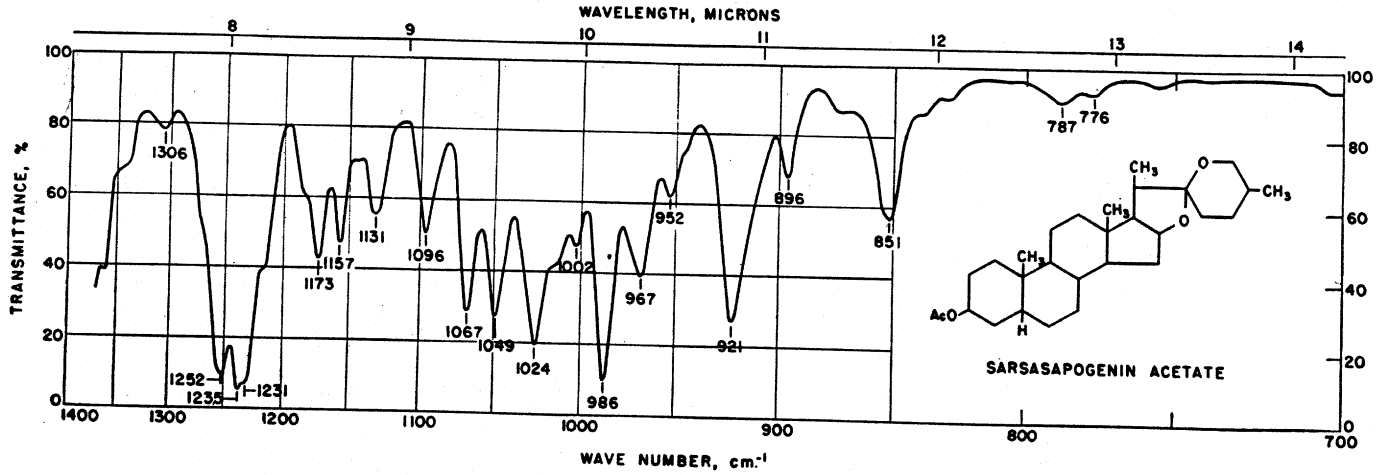


**Figure 9. Sarsasapogenin Acetate (Spirostan-3β-ol 3-Acetate)**

10.0 grams per liter in $CS_2$. 1.1-mm. cell

cm.$^{-1}$ band is not exceptionally strong in the closed-ring sapogenin ketals.

### IDENTIFICATION OF A SAPOGENIN

An unknown sapogenin acetate can be readily identified by means of these curves by the following procedure.

A. First examine the spectrum for the presence of the four absorption bands near 860, 900, 920, and 980 cm.$^{-1}$ If any one of the four is missing, the substance (like kryptogenin) is not a true steroidal sapogenin with an *F* ring. If the bands occur at 850, 900, 920, and 986 cm.$^{-1}$, with the 920 band stronger than the 900 band, it is a sapogenin with "normal" configuration of the *F* ring. Compare with the rest of the bands of Figure 9 to determine whether it is sarsasapogenin (the most common "normal" plant sapogenin). If the four bands occur at 865, 900, 920, and 981 cm.$^{-1}$, with the 900 band stronger than the 920 band, the substance is a sapogenin with "iso" configuration of the *F* ring.

B. Determine whether the unknown is the acetate of a monoor of a dihydroxy sapogenin by examining the acetate bands near 1240 and 1740 cm.$^{-1}$ Figures 2, 4, 9, 10, and 11 show the strength of these bands typical of monohydroxy sapogenin acetates. Figures 1, 3, 5, 6, 7, 8, and 12 show the much stronger acetate bands typical of sapogenin diacetates.

C. If the preceding examination indicates a monohydroxy sapogenin, distinguish among diosgenin, hecogenin, smilagenin, and tigogenin by examining details of the fingerprint. Diosgenin is identified by prominent bands at 813 and 838 cm.$^{-1}$ (possibly associated with a $\Delta^5$ ethylenic bond). Hecogenin is identified by its carbonyl band at 1712 cm.$^{-1}$ Smilagenin is identified by the multiple nature of the band between 1200 and 1260 cm.$^{-1}$ and by its unusually high transmittance at 1034 cm.$^{-1}$ Tigogenin is identified by the band at 995 cm.$^{-1}$ and by the fact that it has strong bands at both 960 and 1025 cm.$^{-1}$

D. If the examination under B indicates a dihydroxy sapogenin, distinguish among chlorogenin, gitogenin, kammogenin, manogenin, samogenin, and yuccagenin by the following details: Chlorogenin is identified by the band at 1198 cm.$^{-1}$ and by the two strong bands of nearly equal strength at 1028 and 1055, with a high transmittance maximum between them at 1041 cm.$^{-1}$ Gitogenin is identified by the general appearance of the band between 1030 and 1060 cm.$^{-1}$ and by the two weak bands at 913 and 935 cm.$^{-1}$ Kammogenin is identified by its carbonyl band at 1714 cm.$^{-1}$ and the system of bands at 810, 826, and 845 cm.$^{-1}$ Manogenin is identified by its carbonyl band at 1714 cm.$^{-1}$ and absence of bands between 800 and 850 cm.$^{-1}$ Samogenin is identified by the resolved acetate band at 1221 cm.$^{-1}$ and by bands at 940 and 1145 cm.$^{-1}$ Yuccagenin is identified by the strong band at 911 cm.$^{-1}$ and the bands at 825 and 843 cm.$^{-1}$

E. Confirm the identification by comparing the spectrum of the unknown with the entire fingerprint spectrum of the corresponding reference compound. The two must agree at every absorption band, not only in wave number but also in relative intensity.

Occasionally other sapogenins are found in plant extracts besides those listed here but have not as yet been isolated in sufficiently pure form by the authors to warrant publication of

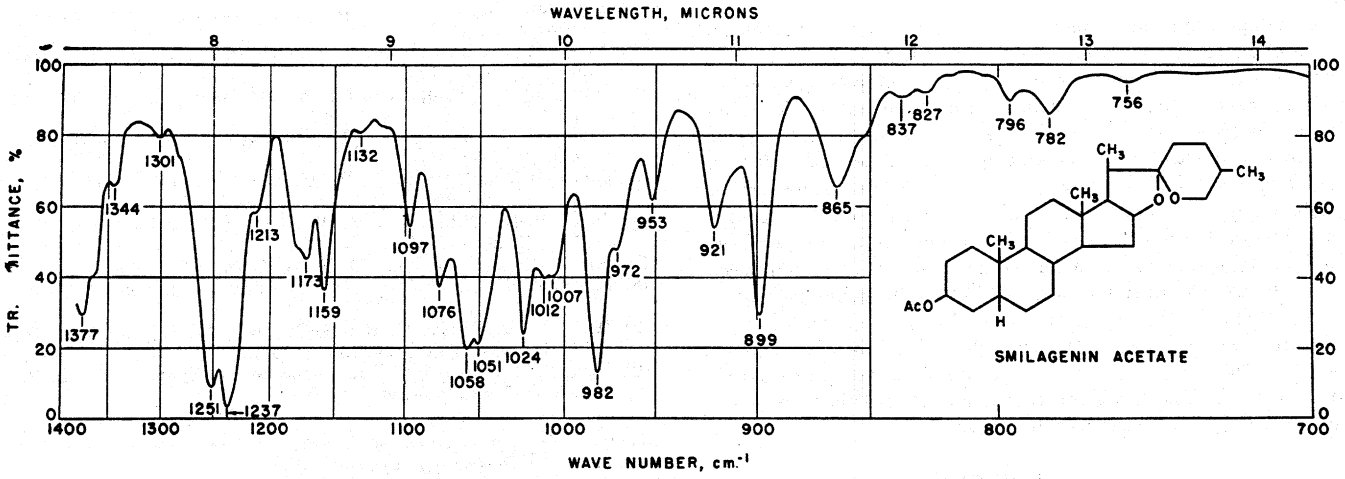


**Figure 10. Smilagenin Acetate (22-Isospirostan-3β-ol 3-Acetate)**

**10.0 grams per liter in $CS_2$. 1.1-mm. cell**

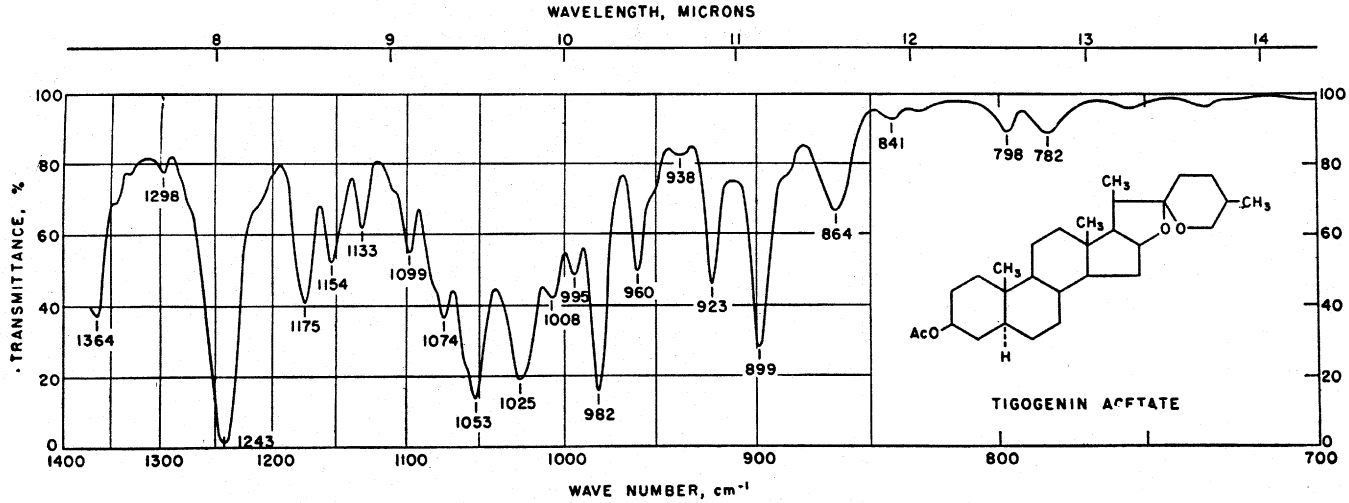


**Figure 11. Tigogenin Acetate (22-Isoallospirostan-3β-ol 3-Acetate)**

**10.0 grams per liter in $CS_2$. 1.1-mm. cell**

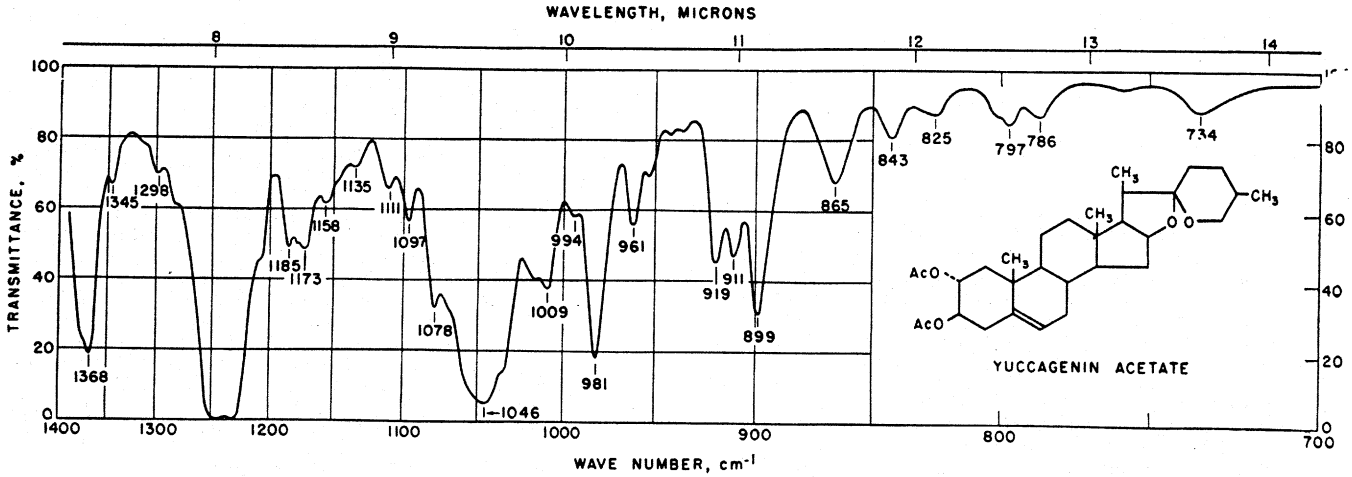


**Figure 12. Yuccagenin Acetate ($\Delta^5$-22-Isospirosten-2$\alpha$,3$\beta$-diol 2,3-Diacetate)**

**10.4 grams per liter in $CS_2$. 1.1-mm. cell**

their spectra. Two of them can be readily identified by the following characteristics: 9-Dehydrohecogenin can be identified by iso configuration from A, monohydroxy from B, $\alpha,\beta$-unsaturated ketonic band at 1680 cm.$^{-1}$ 9-Dehydromanogenin can be identified by iso configuration from A, dihydroxy from B, $\alpha,\beta$-unsaturated ketonic band at 1681 cm.$^{-1}$

A similar systematic identification procedure can be set up for the unacetylated sapogenins in chloroform solution. The spectral tests for *F* ring, normal *vs.* iso configuration, carbonyl, and $\Delta^5$ unsaturation are the same as for the acetates. The distinction between mono- and dihydroxy sapogenins is not as clear cut as with the acetates, but can usually be made in the 1000 to 1100 cm.$^{-1}$ region. In general, monohydroxy sapogenins have a single band near 1050 cm.$^{-1}$, whereas dihydroxy sapogenins have a band that is stronger, broader, or more complex. If these preliminary tests point to a known genin whose spectrum turns out to be substantially identical to that of the unknown, and if the spectrum of the unknown is clearly different from any of the other known spectra, the identification is considered satisfactory. On the other hand, if the sample is not pure enough to allow a decision among the known spectra, it is best to acetylate and compare with the acetate curves.


## ACKNOWLEDGMENT

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