[CONTRIBUTION FROM THE LABORATORY OF BOTANICA-MEX., S. A.]

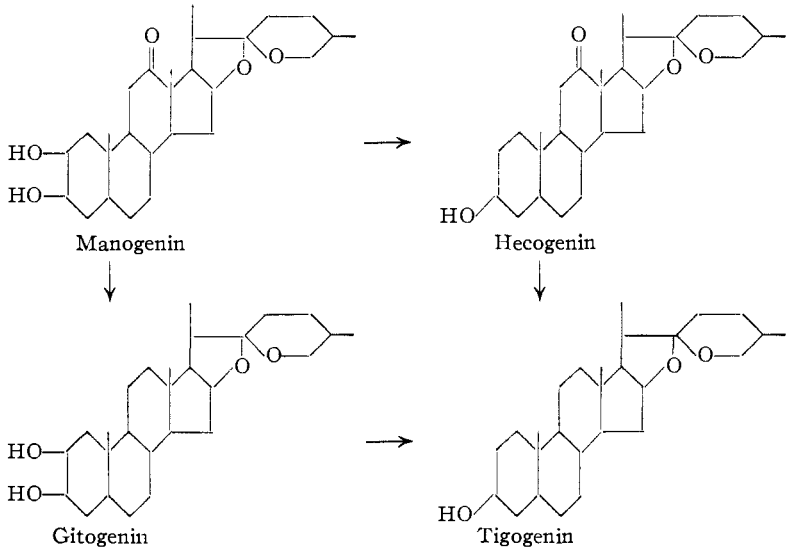
Steroidal Sapogenins. No. 171. Biogenesis of the Steroidal Sapogenins in Agaves, Manfreda and Hesperaloe

BY RUSSELL E. MARKER[1] AND JOSEFINA LOPEZ

We have previously shown that very young plants of *Agave parassana* contained only manogenin as their steroidal sapogenin,[2] whereas when the plant was at its fruiting stage this sapogenin had entirely disappeared and in its place was a mixture of the simpler sapogenins, hecogenin, gitogenin and tigogenin.

We have now extended this study of the biogenesis of the steroidal sapogenins to five other species of Agaves, a Manfreda and a Hesperaloe, and find that all of these plants exhibit the same biogenetic transformation of the more complex sapogenins into the simpler ones as maturity approaches.

Previously it was reported on a preliminary study[3] that *Agave atrovirens* contained manogenin only; *Agave bracteosa*, manogenin; *Agave endlichiana*, hecogenin; *Agave stricta*, gitogenin and tigogenin; *Agave mitraeformis*, manogenin; *Manfreda gigantea*, gitogenin; and *Hesperaloe funifera*, hecogenin. A careful study of the sapogenins present in the very young plants of the above species has been made as well as those in the fruiting stage. For this work the entire plant was used and the young plants were collected at the same time and in the same locality as the mature plants. It was found that the only sapogenin present in the young plants of all the above species was manogenin, but when the plant was at the fruiting period manogenin was absent and in its place was a mixture of the simpler steroidal sapogenins, hecogenin, gitogenin and tigogenin.



The above formulas show the biogenetic course that the sapogenins take in the transformation of the more complex manogenin into hecogenin, gitogenin and tigogenin. This confirms our previous reports on the biogenesis of the more complex sapogenins into the simpler ones,[2] and shows a necessity for a consideration of the age cycle of the plant in making a study of the sapogenins present in the plant.

Experimental Part

A separate collection of very young plants and plants which contained the green fruit was made at the same time and from the same locality. The entire plant was used. The plants were shredded, dried and pulverized. In each case 10 kg. of the dried plant was extracted with alcohol and the extract was concentrated to about 5 liters. To this was added 1 liter of concentrated hydrochloric acid and the mixture was refluxed for three hours. The solution was cooled and extracted well with ether. The ethereal solution was washed with sodium hydroxide solution and the solvent was removed.

Sapogenins from Agave atrovirens, Agave bracteosa, Agave endlichiana, Agave stricta, Agave mitraeformis, Manfreda gigantea and Hesperaloe funifera. Young Plants.—In each species of plant the sapogenin fraction was treated with Girard reagent to remove the ketonic sapogenins. These were crystallized from ether and gave melting points in the range of 252–254°. A mixed melting point with manogenin gave no depression. Yields were 10.3–22.8 g.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.9; H, 9.6.

Acetylation and crystallization from acetic anhydride and from methanol gave manogenin diacetate, m. p. and mixed m. p. 242°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 69.9; H, 8.8.

No other sterols could be isolated from the young plants.

Plants at Fruiting Period.—The sapogenin fraction from each of the mature plants was treated in alcohol with Girard reagent to separate into a ketonic and a non-ketonic fraction. The ketonic fraction was acetylated and the product was crystallized from methanol giving melting points in the range of 241–243°. A mixture with hecogenin acetate gave no depression. Yields were 2.6–4.8 g.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.7; H, 9.6.

Hydrolysis with alcoholic potassium hydroxide and crystallization from ether gave hecogenin, m. p. and mixed m. p. 255–257°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.4; H, 9.9.

(1) Present address: Hotel Geneve, Mexico City.

(2) Marker and Lopez, THIS JOURNAL, 68, 2380 (1947).

(3) Marker and co-workers, *ibid.*, 65, 1206 (1943).

The non-ketonic fractions were washed with a small amount of ether to remove oily products. They were then crystallized from methanol to give products melting in the range of 205–208°. A mixture with tigogenin gave no depression. Yields were 8.3–14.7 g.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 78.0; H, 10.6.

Acetylation and crystallization from methanol gave tigogenin acetate, m. p. and mixed m. p. 204°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.1; H, 9.9.

The mother liquors from the crystallization of tigogenin were concentrated and the residue was crystallized from ether to give gitogenin, m. p. and mixed m. p. 266–268°. Yields were 4.6–8.1 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.0; H, 10.1.

Acetylation and crystallization from methanol gave gitogenin diacetate, m. p. and mixed m. p. 242°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.1; H, 9.6.

No other steroidal sapogenins could be isolated from the mature plants.

Summary

Young plants of *Agave atrovirens, Agave bracteosa, Agave endlichiana, Agave stricta, Agave mitraeformis, Manfreda gigantea* and *Hesperaloe funifera* gave only manogenin as their steroidal sapogenin. When the plants reached maturity this was converted into hecogenin, gitogenin and tigogenin.

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The Bromination of 3-Ketosteroids in Acetic Acid and the Effect of Trace Substances in the Solvent

BY CARL DJERASSI AND CAESAR R. SCHOLZ

Recently, it was noted[1] that the course and the rate of the dibromination of androstan-17α-ol-3-one 17-hexahydrobenzoate (Ia) in acetic acid was dependent to a large extent on whether the acetic acid had been distilled from permanganate. No details were given. Since brominations are used widely in steroid chemistry and are generally performed in acetic acid, we have studied more closely the effect of trace substances in the acetic acid on the bromination of four 3-ketosteroids of the *allo* series.

Since it had been found[1] that the rotations of the two isomeric dibromo ketones IIIa and IVa differed widely, it was possible to follow the course of the dibromination of the ketone Ia polarimetrically, similar to the determination of the mutarotation of sugars. The results of the polarimetric studies are summarized in Fig. 1 and Table I. Using C. P. glacial acetic acid, it was found that within five minutes the rotation of the solution rose to a maximum of +110° at the time of decolorization and then the rotation receded. The time required to reach this point of regression gave a quantitative measure of the rate of dibromination. Raising the temperature or adding hydrogen bromide caused the bromination to proceed so rapidly that this maximum could not be observed (*cf.* Fig. 1). However, when acetic acid was used which had been distilled from potassium permanganate or chromic trioxide, the bromination was very slow and nearly four hours were required for the rotation to reach its maximum value. As was to be expected, the addition of hydrogen bromide increased the rate of bromination, but more hydrogen bromide had to be added to obtain the same results as with C. P. acetic acid.

(1) Wilds and Djerassi, THIS JOURNAL, **68**, 2125 (1946); *cf.* footnote 22a.

The polarimetric study also afforded a means of following the rate of rearrangement of the 2,2-dibromo ketone IIIa to the 2,4-isomer IVa. In C. P. acetic acid, the "half life"[2] of the 2,2-dibromo compound IIIa was approximately one hour, but could be decreased enormously by raising the temperature or by adding hydrogen bromide. In acetic acid distilled from permanganate, the rate of rearrangement was very slow (half life of *ca.* eighteen hours); this behavior could be duplicated in C. P. acetic acid which had not been distilled from permanganate by adding sodium acetate or 30% hydrogen peroxide at the end of the bromination.

On first thought, it appeared that small amounts of peroxide or peracid were formed during the oxidative treatment, which removed some of the hydrogen bromide liberated and thus slowed the rate of bromination and rearrangement. This possibility was excluded, however, by the observation that addition of reducing agents such as Raney nickel or sodium bisulfite to the acetic acid, followed by distillation, did not remove the inhibitor, nor was it affected by long refluxing or several distillations. Furthermore, the presence of peroxide could not be demonstrated iodometrically. The addition of small amounts of heavy metal salts, especially those of chromium and manganese to C. P. acetic acid had no definite effect on the rate of reaction and thus eliminated the possibility that some of the oxidizing agent was carried over into the distillate. The problem was finally solved when C. P. acetic acid and C. P. acetic acid distilled from permanganate was fractionated through a thirty-plate column. These results are summarized in Table I. The impurity was con-

(2) The term "half life" denotes the time required for the rotation of the solution to drop from +110 to +58°, since the total change in rotation is 116° (+110 to −6°).