STEROIDAL SAPOGENINS. V.[1] ENZYMATIC HYDROLYSIS OF STEROIDAL SAPONINS

Sir:

It is well known that plants containing cardio-active glycosides have enzyme systems which can partially hydrolyze these substances.[2] We have recently found that leaves of some Agave and Yucca species which contain steroidal saponins have similar enzyme systems. In contrast to the enzymes acting on cardio-active glycosides, those found in the sapogenaceous plants tested cleave the saponin substrate to the sapogenin aglycone and component sugars or polysaccharides.

An enzyme preparation was obtained by grinding 2 kg. of frozen *Agave toumeyana*[3] leaves, followed by extraction with water at 10°. The filtered extract was shaken with benzene I and the aqueous layer containing the enzyme fraction held at 4°. The leaf residue remaining from the aqueous extraction was refluxed with 95% ethanol to extract residual saponins. The ethanol solution was concentrated, diluted with water, extracted with benzene II, and the aqueous saponin fraction heated on the steam bath to drive off residual alcohol.

The aqueous enzyme and saponin solutions were combined, adjusted to *p*H 5.25 and held at 37° for 90 hours. The resultant turbid suspension was extracted first with a mixture of 90% benzene–10% ethanol III to recover enzyme hydrolyzed sapogenins and then with butanol IV to obtain unhydrolyzed or partially hydrolyzed saponins.

Only a trace of sapogenin was found in the prehydrolysis benzene extracts I and II. The extract III was concentrated and chromatographed on activated alumina. Using methods presented in detail elsewhere[4] the following sapogenins were isolated: (1) hecogenin (isoallospirostan-12-one-3β-ol), m.p. Kofler 255–260°, yield 3.9 g., on Wolf–Kishner reduction gave tigogenin (isoallospirostan-3β-ol) m.p. 203–205°; (2) manogenin (isoallospirostan-12-one-2α,3β-diol) m.p. 240–244°, yield 4.3 g., on Wolf–Kishner reduction gave gitogenin (isoallospirostan-2α-3β-diol) m.p. 268–270°. Infrared spectra of all sapogenins and their Wolf–Kishner reduction products were identical (with exception of manogenin which showed slight divergencies) with those of authentic specimens. Hecogenin and manogenin had a carbonyl peak in the infrared at 1708–1710 cm.$^{-1}$, absent in reduced products. Manogenin also showed conjugated carbonyl at 1678 cm.$^{-1}$ from which it is deduced that 10–20% of the 9$^{(11)}$ dehydro component was present.

From IV after subsequent acid hydrolysis and chromatography were isolated 2.4 g. of sapogenin similar in composition to those found in III, indicating that the enzymatic hydrolysis was 78–80% complete under our experimental conditions.

In a duplicate experiment, the sapogenins were isolated entirely by means of acid hydrolysis. The yield of total sapogenin was similar and the sapogenins isolated identical with the enzymatic hydrolysis.

Using methods similar to those described above, acid or enzyme hydrolysis of *Agave serulata*[5] gave hecogenin, manogenin and small quantities of the 9$^{(11)}$-dehydro analogs of both sapogenins; an unidentified Yucca species[6] yielded with both methods sarsasapogenin (spirostan-3β-ol) and an unidentified Dioscorea species gave diosgenin (Δ^5-isospirosten-3β-ol) in both cases.

In agreement with Marker, *et al.*,[7] we find that steroidal sapogenins occur in plants only as glycosides as evidenced by the fact that free sapogenin could not be found prior to hydrolysis. Our data are not in accordance with the views of Marker and Lopez[8] that the spiroketal side chain and the 12-keto group in sapogenins are artifacts produced by acid hydrolysis. The fact that the much milder enzyme hydrolysis gives the same products as acid cleavage tends to cast doubt on Marker's hypothesis. Our previous findings[9] based on infrared studies of saponins have also shown that the structure of the steroidal moiety of saponins and their sapogenins derived by acid hydrolysis are in all probability identical.

Because of the mild conditions used, tars, resins and pigments produced by acid hydrolysis are not found in the enzymatic method, thus simplifying the isolation of purified sapogenins.

The distribution, occurrence, and physico-chemical properties of the enzyme systems found in sapogenaceous plants are being studied and will be reported in detail at a later date.

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(2) For pertinent references see A. Stoll and associates, *Helv. Chim. Acta*, 1933–1951, and W. A. Jacobs, *Physiol. Rev.*, **13**, 222 (1933).

(3) Collected by H. S. Gentry, #S 2046, Superior, Arizona, December, 1951.

(4) M. E. Wall, M. M. Krider, E. S. Rothman and C. R. Eddy, *J. Biol. Chem.*, in press.

(5) Collected by H. S. Gentry, #1865, San Ignacio, Baja California, November, 1951.

(6) Collected by H. S. Gentry, #2161, Jecabasco, Mexico, December, 1951.

(7) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(8) R. E. Marker and J. Lopez, *ibid.*, **69** 2390 (1947).

(9) E. S. Rothman, M. E. Wall and H. Walens, *ibid.*, in press.