dioxolane ring is supposed to be a typical form of all such rings which are postulated as intermediates in certain well-known molecular rearrangements and group migrations, and explains the facility with which these transitions occur.[22]

The relative stability of γ- and δ-lactones would appear to be definitely an exception to this rule. There is some evidence that the carbon valence angle in the carbonyl group of ketones is about 132°. If this be so, then the greater stability of γ-lactones finds a satisfactory explanation, since the increased magnitude of the carbon valence angle cancels the effect of the decrease in the oxygen angle to about 90°. This suggestion, however, does not explain the relative instability of δ-lactones any more than the Sachse–Mohr theory. A possible explanation has been offered by Carothers[31] in connection with the remarkable tendency toward polymerization shown by certain six-membered cyclic esters.

It would appear that many of the properties of the oxide rings of carbohydrates and polysaccharides with respect to stability, ease of formation, and interconvertibility, can be explained on the basis that the oxygen valence angle is about 90°.

Acknowledgment.—We take this opportunity to acknowledge the kindness of Dr. Wallace H. Carothers of E. I. du Pont de Nemours and Company for the gift of the pentamethylene bromide and to thank Dr. Walter Mitchell of this Department for the preparation of the tetrahydropyran.

(31) Carothers, *Chem. Reviews*, **8**, 406 (1931).

Summary

1. Trimethylene oxide and tetrahydropyran have been prepared, their densities and the dielectric constants of their dilute solutions, and those of propylene oxide measured. The electric moments are 2.01, 1.87 and 1.88 $\times$ 10^{18} e. s. u., respectively.

2. The "normal" oxygen valence angle has been determined for tetrahydropyran as 90 $\pm$ 5°. The "normal" angle is defined as that assumed by the valence bonds under ideal *intramolecular* conditions.

3. The value of the moment of propylene oxide supports the conclusions that substitution of a hydrogen atom by a methyl group causes no change in moment in certain homologous series. The values of the oxygen valence angle in some heterocyclic rings have been calculated and tabulated.

4. The electric moments of the monochloroacetate I and the cyclic form of the dichloroacetate (2-hydroxy-2-dichloromethyl-1,3-dioxolane) II are 3.94 and 3.35 $\times$ 10^{-18} e. s. u., respectively. The difference in the moments is explained by the formation of the dioxolane ring structure.

MONTREAL, CANADA RECEIVED APRIL 6, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

Saponins and Sapogenins. I. Echinocystic Acid

BY I. BERGSTEINSSON AND C. R. NOLLER

Several species of plants belonging to the gourd family and native to the Pacific Coast are commonly called big-root, man-root, man-in-the-ground, wild cucumber or Chilicothe vine. Jepson[1] states that *Echinocystis marah* (*Megarrhiza marah*) is to be found in the hills of Marin, Alameda and Contra Costa counties and northward, *E. fabacea* (*M. californica*), the most common species, in the Coast Ranges and the Sacramento and San Joaquin valleys, *E. watsonii* in the Sierra Nevada and *E. macrocarpa* from the Kaweah River basin to Southern California.

As the common names imply, all of these species are especially characterized by a huge root weighing up to perhaps fifty kilos which can be readily imagined to resemble the body of a man. The portion above ground is a rather graceful vine which bears green round, or oval fruit with soft spines. It is likely that the roots of all species have been used as fish-poisons by the California Indians. Thus Chestnut[2] states that the Indians of Mendocino County used the root of *Magarrhiza marah* for this purpose and as a medicine. Extracts of one of the species, prob-

(1) W. L. Jepson, "Flora of Western Middle California," Cunningham, Curtiss and Welch, San Francisco, 1911, 2d ed., p. 270.

(2) Chestnut, "Contributions from the U. S. National Herbarium," **7**, 390 (1902).

ably *M. marah*, were formerly added to sprays by Oregon orchardists, possibly because of their wetting properties.[3] The mock-orange or Calabazilla (*Curcubita foetidissima*), another member of the gourd family and a native of Southern California, likewise has a huge root which was used as a soap substitute by the Indians and Spanish Californians. One is naturally led to the conclusion that these properties are due to the presence of high concentrations of one or more saponins.

Examination of the root of *E. fabacea* has led to the isolation of a product having all the properties of a saponin. Its aqueous solution foams on shaking, is a potent fish poison and readily causes the hemolysis of red blood corpuscles. Hydrolysis of the saponin yields a crystalline acid sapogenin which appears to be different from any recorded in the literature and which we have named "echinocystic acid." Analysis of the acid and its derivatives indicates an empirical formula of $C_{30}H_{48}O_4$. The presence of a carboxyl group is shown by titration with standard alkali and by the fact that a methyl ester can be prepared using either diazomethane or methyl sulfate and alkali as a methylating agent. The other two oxygen atoms are accounted for by two hydroxyl groups as shown by the preparation of a diacetate and a diacetate methyl ester.

As is the case with most of the acid sapogenins and triterpenoids, the methyl ester resists hydrolysis by the usual methods. From the data on the rate of saponification of the diacetate it appears that one of the acetyl groups is hydrolyzed much more slowly than the other. Thus an amount of alkali equivalent to one acetyl group is used up in approximately ten hours, whereas reaction for an additional thirty-eight hours removes only a third of the remaining acetyl. Similar results have been reported for betulin diacetate[4] and indicate that one of the hydroxyl groups is primary and the other secondary.

A solution of echinocystic acid or its diacetate in carbon tetrachloride gives a yellow color with tetranitromethane, indicating the presence of at least one double bond. Neither the acid nor its diacetate can be hydrogenated using platinum oxide catalyst in acetic acid solution, nor can a pure product be obtained from the action of bromine on an alcoholic solution of the acid.[5]

(3) Private communication from Mr. Stanley Knapp.
(4) R. Vesterberg, *Ber.*, **65**, 1305 (1932).
(5) *Cf.* Winterstein, *Z. physiol. Chem.*, **199**, 34, 41, 46 (1931).

Assuming the data recorded in the literature and those of the present work to be correct, echinocystic acid appears to be different from any sapogenin or triterpenoid previously reported. Of the better characterized acid triterpenoids, it appears to be isomeric with sumaresinolic acid[6] or possibly with siaresinolic acid[6] or hederagenin.[6,7] Due to the difficulties of analysis encountered with this class of compounds the data in the literature are often conflicting and it is impossible to be definite in this respect. From the fact that the methyl esters of these acids are all hydrolyzed with difficulty it seems likely that the carboxyl group occupies a similar position in all of them. Echinocystic acid does not form an acetonyl derivative nor could a pure compound be isolated from its reaction with thionyl chloride, proving that the two hydroxyl groups react differently than those in hederagenin[8] and indicating that they are not in 1,2 or 1,3 positions with respect to each other. The ready formation of a diacetate shows that the hydroxyl groups bear a different relationship to the rest of the molecule than those of siaresinolic[9] and sumaresinolic[10] acids since these acids yield with difficulty only monoacetates.

Assuming a five ring system, which seems fairly well established for the triterpenoids,[11] and the presence of one double bond, echinocystic acid is best represented by the formula $C_{27}H_{42}(CHOH)(CH_2OH)COOH$.

Experimental

Isolation of the Saponin.—A supply of the roots of *Echinocystis fabacea* was gathered in the spring shortly after they had sprouted, and stored in a dark, dry room. In a typical extraction, 1 kg. of finely ground root was refluxed for two hours with 1.5 liters of methyl alcohol and the hot extract decanted. A second similar extraction was made with 1.5 liters of 50% aqueous methyl alcohol and a final extraction with straight methyl alcohol. The combined extract was evaporated to 2 liters and filtered, giving a light yellow solution. One liter of this solution evaporated to dryness at 90° to constant weight gave 45 g. of crude saponin. The dry powder causes sneezing and is extremely bitter. Its aqueous solution forms stable foams on shaking, causes hemolysis and is toxic to goldfish.

Isolation of the Sapogenin, Echinocystic Acid.—To a solution of 45 g. of crude saponin in 890 cc. of water and 1200 cc. of methyl alcohol was added 400 cc. of concd. hydro-

(6) Ruzicka and Furter, *Helv. Chim. Acta*, **15**, 472 (1932).
(7) Winterstein and Stein, *Z. physiol. Chem.*, **211**, 5 (1932).
(8) Jacobs, *J. Biol. Chem.*, **63**, 631 (1925).
(9) Winterstein and Egli, *Z. physiol. Chem.*, **202**, 207 (1931).
(10) Private communication from Dr. H. Hösli.
(11) Ruzicka, Brüngger, Egli, Ehmann, Furter and Hösli, *Helv. Chim. Acta*, **15**, 431, 1496 (1932).

chloric acid and the solution heated at 60°. After one hour, the gelatinous prosapogenin separated and after sixty hours this was converted into the white granular sapogenin. This was filtered from the dark brown solution, which on concentration and cooling gave a second crop of crystals. The total yield of crude sapogenin melting at 295–300° was 4.8 g. For the preparation of larger quantities of sapogenin the original extracts of the root were hydrolyzed without isolating the saponin by adding sufficient sulfuric acid to give a 7% solution and refluxing.

The sapogenin is practically insoluble in cold or hot benzene, ligroin and carbon tetrachloride. It is slightly soluble in hot chloroform, from which it separates as a gel on cooling. It is readily soluble in cold acetone and can be recrystallized from ether, acetic acid, ethyl acetate and the alcohols. From methyl or ethyl alcohols it crystallizes slowly as fibrous needles and from isopropyl alcohol as glistening plates containing alcohol of crystallization. Isopropyl alcohol has less tendency to form supersaturated solutions and removes the color more readily than methyl or ethyl alcohol. The alcohol of crystallization is lost only slowly at 110° under a high vacuum so that for analysis the sample was dried at 175° at 1–2 mm. The product after five crystallizations has a constant rotation and melts at 305–312° (corr.) with decomposition. Loss of carbon dioxide begins to take place at about 250° so that the high melting points are obtained only in a preheated bath: $[\alpha]^{28}_{546}$ +40.6° in 95% alcohol.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24; neut. equiv., 472.4. Found: C, 76.13, 75.94, 76.00, 76.18; H, 10.84, 10.91, 10.71, 10.88; neut. equiv., 474.3, 472.6, 475.3, 475.8, 475.9.

Methyl Echinocystate.—To 6 g. of sapogenin in ether was added an ether solution of diazomethane until a slight yellow color persisted. After standing overnight the ether was evaporated. The residue was crystallized six times from methyl alcohol, when it melted at 213–215°. For analysis the sample was dried in a high vacuum at 110°: $[\alpha]^{28}_{546}$ 37.08 in 95% alcohol.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.48; H, 10.36. Found: C, 76.31, 76.37, 76.64, 76.68; H, 10.37, 10.40, 10.43, 10.56.

For the preparation of large quantities of the methyl ester the use of methyl sulfate as described by Jacobs[8] for preparing hederagenin methyl ester is quite satisfactory. In fact the crude ester is somewhat purer than that prepared from diazomethane. The methyl ester is recovered unchanged after boiling for one hour with alcoholic potassium hydroxide solution.

Echinocystic Acid Diacetate.—A solution of 25 g. of sapogenin in 400 cc. of glacial acetic acid, 50 cc. of acetic anhydride and 2 g. of fused sodium acetate was refluxed for two hours. The volume was reduced to 75 cc. by distillation at 20 mm., and the excess acetic anhydride destroyed by adding 100 cc. of methyl alcohol and refluxing. On repeated concentration and removal of the product by filtration, a total of 23 g. of crude diacetate was obtained. It is only slightly soluble in ligroin, readily in chloroform, and fairly soluble in benzene, carbon tetrachloride, acetone, ether and the alcohols. For analysis it was crystallized five times from methyl alcohol and dried in a high vacuum at 110°, when it melted at 272–275° (corr.); $[\alpha]^{27}_{546}$ −14.6° in chloroform.

Anal. Calcd. for $C_{34}H_{52}O_6$: C, 73.33; H, 9.42; neut. equiv., 556.4. Found: C, 73.16, 72.95, 72.64, 73.03; H, 9.80, 9.61, 9.77, 9.64; neut. equiv., 556.8, 557.4.

Saponification of the Diacetate.—A series of flasks containing aliquot portions of an alcoholic solution of a weighed amount of the diacetate and an excess of standard potassium hydroxide solution were heated in the same bath at 60°. From time to time a flask was removed and the contents titrated with standard alkali. The percentage of complete hydrolysis was as follows.

Elapsed time, hours	1	3.5	7	12	24	48
Hydrolysis, %	13.7	31.1	45.9	54.6	61.2	66.3

Thus sufficient alkali was used to remove one acetyl radical at the end of about ten hours but only a third of the remaining acetyl radical is removed at the end of forty-eight hours.

Methyl Echinocystate Diacetate.—A solution of 10 g. of the diacetate in ether was esterified with diazomethane, 100 cc. of methyl alcohol and 5 cc. of water added, and the solution concentrated on the water-bath. A total of 9 g. of ester was obtained which melted at 199–201° (corr.). The methyl ester is slightly less soluble in most solvents than the acid diacetate. It was crystallized four times from methyl alcohol and dried at 110° in a high vacuum for analysis. It melted at 200–201°; $[\alpha]^{28}_{546}$ −15.1° in chloroform.

Anal. Calcd. for $C_{35}H_{54}O_6$: C, 73.63; H, 9.54. Found: C, 73.25, 73.22; H, 9.50, 9.57.

The product obtained by acetylation of methyl echinocystate had the same melting point as the above compound and the mixed melting point showed no depression.

Summary

A new saponin and crystalline sapogenin have been isolated from *Echinocystis fabacea*. The sapogenin, which has been named echinocystic acid, is acidic and contains two hydroxyl groups, one of which appears to be primary and the other secondary. It has been converted into a methyl ester, a diacetate and methyl ester diacetate and analyses indicate that it is a triterpenoid with the probable formula $C_{27}H_{42}(CHOH)(CH_2OH)COOH$.

STANFORD UNIVERSITY, CALIFORNIA

RECEIVED APRIL 6, 1934