TABLE I

IDENTIFICATION CHARACTERISTICS OF SAPOGENINS USED IN THIS STUDY

Sapogenin	Melting point, °C.[a] Genin	Acetate	Specific rotation[b] Genin	Acetate	Infrared absorption
Tigogenin	207–209	205–206	−70	−74	Carbonyl absent
Hecogenin	260–261	245–246	+ 7	− 5	Carbonyl max. at 1706 cm.$^{-1}$
Kammogenin	241–243	253–254	−54	−80	Carbonyl max. at 1714 cm.$^{-1}$
Manogenin	244–246	248–250	− 2	−42	Carbonyl max. at 1709 cm.$^{-1}$
9,11-Dehydromanogenin[c]	232–233	258–260	− 7.8	..	Carbonyl max. at 1676 cm.$^{-1}$ C=C max. at 1602 cm.$^{-1}$

[a] All melting point determinations made with the Kofler block. [b] Rotations determined at 25°, sodium lamp, concentrations between 8–10 mg./ml. [c] 95% pure, as estimated from ultraviolet and infrared absorption, with approximately 5% of a non-conjugated carbonyl sapogenin.

are important as precursors in steroid syntheses. The present method for the determination of polarographic behavior of these compounds allows the use of a non-aqueous medium consisting of a lithium chloride methanol–benzene electrolytic solution in which the 12-ketosapogenins are soluble.

Experimental

A Sargent Model XXI Polarograph was used to obtain the current-voltage curves. The capillary had t and m values of 1.35 seconds and 3.587 mg. per sec., respectively, which gave a capillary constant of 2.46 mg.$^{2/3}$sec.$^{-1/2}$. The m and t values were obtained in an open circuit, with the polarographic cell maintained at 25.0°, and with the capillary dipping into the non-aqueous electrolytic solution. The capillary constant at −1.80 volts under the above conditions was 2.38. This value has been used to calculate the diffusion current constant of the 9,11-dehydromanogenin.

The electrolytic cell was a modified Lingane H-cell[5] with a saturated calomel reference electrode. This cell had an open circuit resistance of 1175 ohms, and all half-wave potentials were corrected for IR drop. Half-wave potential readings were made against the saturated calomel electrode, and the polarograms were obtained at 25 ± 0.1°. The sapogenins were isolated and characterized by methods developed at this Laboratory.[6] Descriptive data for these compounds are presented in Table I. Thirty milliliters of the electrolytic solution, consisting of 0.3 M lithium chloride in a 50–50 (by volume) mixture of absolute methanol–benzene, was measured into the sample arm of the H-cell. The solution was degassed with high-purity nitrogen, and a polarogram was recorded. The sample was then added (10–40 mg.), and the solution was again degassed with nitrogen. A polarogram was recorded, and from the increase in wave height, the diffusion current of the reducible sapogenin was calculated.

TABLE II

POLAROGRAPHIC CHARACTERISTICS OF 9,11-DEHYDROMANOGENIN

Wt. sample per 40 ml.	Mole per liter	i_d, μa.	i_d/C	$i_d/Cm^{2/3}t^{1/6}$
0.0121	0.000885	4.76	5516	2.32
.0258	.001887	10.10	5489	2.31
.0390	.003019	15.70	5643	2.37

Results and Discussion

Tigogenin, which has no keto group, shows no polarographic reduction. Hecogenin and manogenin, both having a 12-keto group but no unsaturated linkage, show no reduction. Kammogenin, which has a 12-keto group and a Δ^5-unsaturated linkage, also does not reduce. However, 9,11-dehydromanogenin, which has both a 12-keto group and a conjugated unsaturated linkage, reduces at the dropping electrode with a half-wave potential of −1.72 volts *vs.* S.C.E. and has a diffusion current constant of 2.33. This diffusion current constant is similar to that found for other conjugated ketones in the non-aqueous electrolyte. Mesityl oxide, for example, has a diffusion current constant of 2.07. The reducibility of the 12-keto group of 9,11-dehydromanogenin in the non-aqueous electrolyte was expected because of the conjugated carbonyl group in this compound. Table II shows that the wave height of the 9,11-dehydromanogenin is directly proportional to concentration in the range studied (10 to 40 mg./30 ml.). The use of the lithium chloride non-aqueous electrolyte made possible the direct polarographic analysis of these water-insoluble steroidal compounds.

Acknowledgment.—The authors acknowledge the assistance of C. R. Eddy in obtaining the infrared data for the sapogenins.

EASTERN REGIONAL RESEARCH LABORATORY[7]
PHILADELPHIA 18, PENNSYLVANIA

---

(5) C. O. Willits, C. Ricciuti, H. B. Knight and D. Swern, *Anal. Chem.*, in press.

(6) M. E. Wall, *et al.*, *J. Biol. Chem.*, in press.

(7) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

The Heat of Neutralization of Hydrogen–Bentonite

BY W. H. SLABAUGH

RECEIVED APRIL 5, 1952

Many clays undergo stoichiometric reactions with various types of reagents. Reactions which involve the base-exchange character of clays have been widely investigated and are adequately reviewed elsewhere.[1] In previous work[2] there was good indication that the exchangeable hydrogen which was present in electrodialyzed bentonite systems was of two principal types, namely, hydrogen ions in the adsorbed layer of the colloidal micelles and hydrogen ions at the base-exchange sites. Further, these ions showed primary and secondary characteristics comparable to a typical diprotic acid. The present work which consists of a thermodynamic study of the neutralization of these hydrogen ions gives good indication that there is a fundamental difference between these types of hydrogen ions and that, during storage, there is a definite tendency for the absorbed ions to become more intimately associated with the clay micelles.

---

(1) E. A. Hauser, *Chem. Revs.*, **37**, 307 (1945); C. E. Marshall, "The Colloid Chemistry of the Silicate Minerals," Academic Press, Inc., New York, N. Y., 1949.

(2) W. H. Slabaugh and J. L. Culbertson, *J. Phys. Colloid Chem.*, **55**, 744 (1951).

Experimental Methods

A 2% suspension of a typical Wyoming bentonite obtained from the Baroid Division of the National Lead Company was permitted to stand for three months. After this time, the supernatant suspension was decanted and electrodialyzed in a Mattson cell from which the so-called hydrogen–bentonite was obtained. Another procedure for the preparation of the acid clay made use of a 30-inch column of Amberlite 120 ion-exchange resin which had previously been converted to its acid form by leaching the column with 10% H_2SO_4.[3] After the column was washed with distilled water until the effluent was neutral, the supernatant clay suspension was passed through the column.

The base-exchange values of this bentonite as determined by titration of these two differently prepared hydrogen-bentonites were 75 milliequivalents per 100 g. of bentonite for the electrodialyzed clay and 106 milliequivalents per 100 g. of bentonite for the columned clay. By the Graham and Sullivan method[4] of $NH_4C_2H_3O_2$ adsorption on the original clay, the base-exchange capacity was 86 milliequivalents per 100 g. of bentonite.

The calorimeter (Fig. 1) which was constructed for this study was contained in a 1-liter dewar flask. A 57-junction copper–constantan thermocouple constructed after the suggestions of White,[5] showed a sensitivity of 2.20 millivolts per degree at 23.5°. In actual operation, the potential produced by the thermocouple was measured by observing the deflection of the galvanometer of the potentiometer. The galvanometer, a Leeds and Northrup Type R, showed a deflection at 1 meter of 4100 mm. for each degree. Over the length of the scale (500 mm.) the relationship between temperature and galvanometer deflection was observed to be exactly linear. Both the potentiometer and heater coil of the calorimeter were operated from heavy duty storage batteries, the potentials of which were essentially constant, during a particular determination. The heater which served to calibrate the calorimeter during each run was a coil of 30 gage constantan wire and had a resistance of 31.65 ohms at 23.5°. The outside end of the thermocouple was immersed in water which was contained in a second dewar flask. The whole assembly was immersed in a 30-liter water-bath which was insulated with 10 cm. of glass wool. The water-bath was controlled to ±0.001° and the whole apparatus was located in a room whose temperature was controlled at 24.5 ± 0.1°.

The over-all reliability and accuracy of the calibrations of this instrument are shown by the results of a study wherein 0.00165 mole of hydrochloric acid in 220 ml. of water reacted with an equivalent amount of sodium hydroxide (10.00 ml. of 0.165 N NaOH). The alkali was added in 1.00-ml. portions and the amount of heat evolved was observed for each of these portions. The actual quantity of heat released in each of these steps was approximately 2.45 calories. The quantity was converted to the corresponding molar heat of neutralization. The mean value for the heat of neutralization of HCl by NaOH based upon these ten determinations was 13,550 ± 195 calories. The probable error was determined by the expression $p = 0.6745\sqrt{\frac{\Sigma\delta^2}{n(n-1)}}$ where p is the probable error of the mean, δ is the deviation of each observation from the mean, and n is the number of observations. In view of the small amount of acid and base involved in these measurements, and the classical values of Richards and Rowe[6] at comparable concentrations (13,640 calories per mole), the accuracy of this method appears adequate.

In making a determination, 220 ml. of a clay suspension was placed in the calorimeter dewar flask and its temperature was adjusted to 23.50 ±0.01°. The reservoir was filled with 0.165 N NaOH at the same temperature, and the calorimeter was assembled and immersed in the water-bath. Both the clay suspension and the bath were adequately agitated with paddle type stirrers. In order to make certain that thermal equilibrium had been attained the calorimeter remained thus for 8 hours before the actual observations were started. Temperature readings as shown by the galvanometer were observed at half-minute intervals in order to establish the slope of the time–temperature curve. One ml. of NaOH was then added by means of the syringe pipet and temperatures were noted until the slope of the time–temperature curve became constant; this usually required about 5 minutes. One-ml. portions of NaOH were repeatedly added until a 50% excess of alkali had been added. Then, three separate calibrations of the heat capacity of the calorimeter were made by passing 0.19 ampere through the heater coil for 10.0 seconds. On occasion, calibrations of the heat capacity were made prior to or during the observations of the neutralization reactions and, in any one run, the calibrations were essentially identical to those obtained at the end of the run. The method used for the calculation of the heat of neutralization from these data is similar to that of Harkins and Dahlstrom.[7] Operation of the syringe and stopcock in the calorimeter produced no detectable quantity of heat.

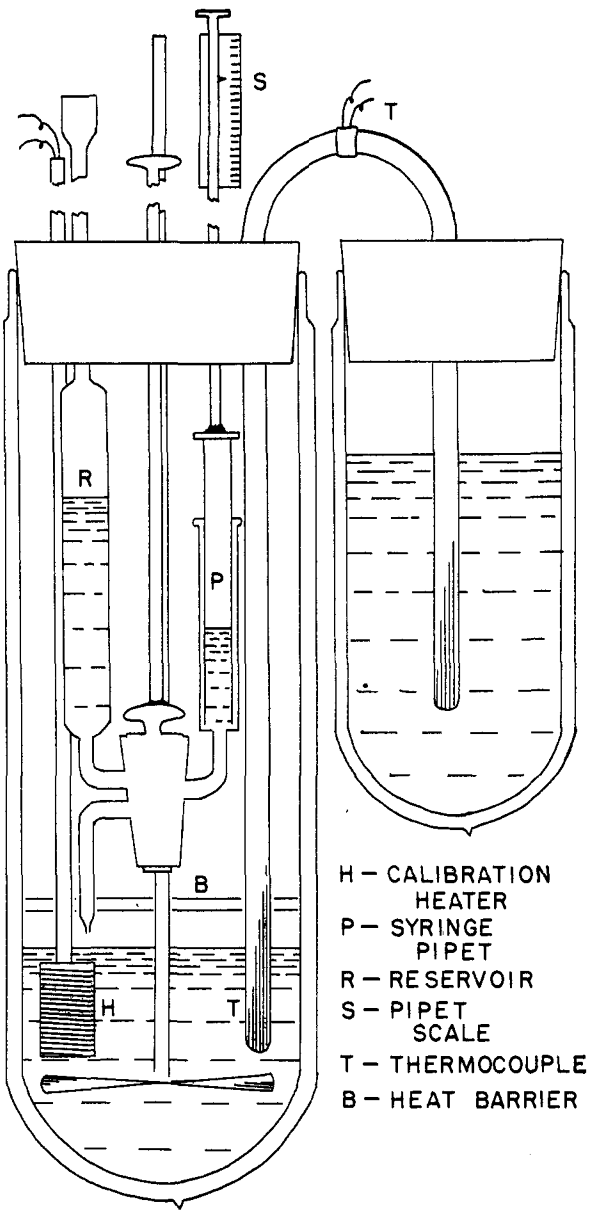

Fig. 1.—The calorimeter for the determination of the step-wise neutralization of hydrogen–bentonite.

All data presented in this paper are the results of duplicate or triplicate runs. The results of the measurements of the heats of neutralization of hydrogen–bentonite are summar-

---

(3) Loren C. Hurd and Wm. L. Van Horne, U. S. Patent, 2,431,481.

(4) Robert P. Graham and J. D. Sullivan, *J. Am. Ceram. Soc.*, **21**, 176 (1939).

(5) W. P. White, THIS JOURNAL, **36**, 1856, 2292 and 2313 (1914).

(6) T. W. Richards and A. W. Rowe, *ibid.*, **44**, 684 (1922).

(7) W. D. Harkins and R. Dahlstrom, *Ind. Eng. Chem.*, **22**, 897 (1930).

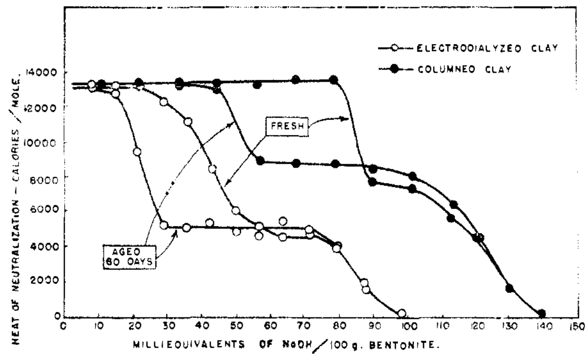

Fig. 2.—Heat of neutralization of electrodialyzed bentonite and bentonite exchanged with hydrogen in an Amberlite IR 120 column.

ized in Fig. 2. The ΔH values were calculated on the basis of molar quantities of alkali added in each step of the neutralization process. For example, when 1 ml. of 0.165 N NaOH (0.0165 millimole) is added to an acid system a certain quantity of heat is evolved. This amount of heat multiplied by $(0.0000165)^{-1}$ gives the amount of heat which would be evolved by the reaction of a mole of NaOH with a given portion of the hydrogen ions in the clay system. The ΔH values do not represent the total heat of neutralization but, rather, the quantity of heat evolved in each successive step in the neutralization process. In plotting these data, the quantity of alkali is expressed in terms of the number of milliequivalents of NaOH per 100 g. of oven-dried bentonite. By determining the total area under the ΔH curves it is possible to express the mean heat of neutralization. The electrodialyzed clay gave values of 8,000 calories per mole for the freshly prepared samples, and 6,700 calories per mole for the aged samples. The columned clay gave values of 10,400 calories and 9,400 calories per mole for the fresh and aged samples, respectively.

In Fig. 3 are the potentiometric titration data for the same system. A glass electrode and a sleeve type calomel cell were used in these titrations.

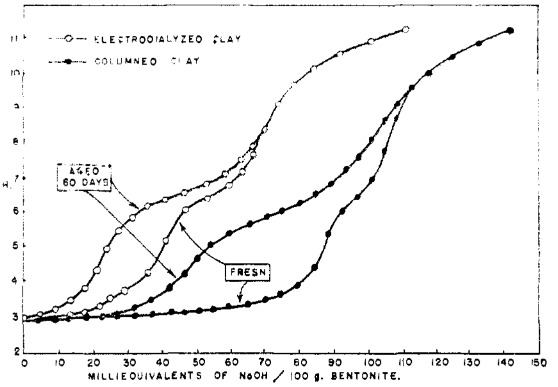

Fig. 3.—Potentiometric titration of electrodialyzed bentonite and bentonite exchanged with hydrogen in an Amberlite IR 120 column.

Discussion

The experimental data obtained in this work show further indication of the existence of two types of available hydrogen in the hydrogen–bentonite system. In the acid-clay from the electrodialysis and column procedures, the early portions of alkali react with hydrogen ions which are completely ionized. During the latter part of the neutralization process, the hydrogen ions show a much different character. The lower heat of reaction shows that these secondary hydrogen ions are less highly ionized, possible less available geometrically, and that considerable energy is required for the hydration of these ions. These two types of hydrogen ions correspond to the plateaus in the ΔH curves.

Considerable heat is evolved by the addition of NaOH beyond the stoichiometric end-point which follows the second plateau in the ΔH curves. This is attributed to the greater degree of covalency of the hydrogen ions which are neutralized only at an alkalinity above pH 9.0. It is doubtful if this heat is associated with the physical adsorption of NaOH which is described by Kayser and co-workers.[8] Apparently, up to 75 milliequivalents of NaOH beyond the stoichiometric end-point can be physically adsorbed. Since this is probably a van der Waals adsorption, the heat of adsorption would likely be too small to detect by the present method.

A significant contrast between the electrodialyzed and columned bentonite is shown by the end-point in the titration curves. For the electrodialyzed clay, the primary end-points require one-half and one-third as much of base as is required for total neutralization of fresh and aged samples, respectively. In the case of the columned clay, the primary end-points require 80 and 50% as much base as is required for the total neutralization of fresh and aged samples, respectively.

Although the total amount of titratable hydrogen ion remains essentially the same, aging produces pronounced effects upon the ratio of primary to secondary hydrogen ions in the clay systems. The curves in Figs. 2 and 3 represent the maximum change in this respect which the hydrogen–clay underwent in 60 days. These data suggest that in the aging process the highly reactive hydrogen ions, which are probably concentrated to a considerable extent in the adsorbed ion layer of the colloidal micelles, migrate into the micelle and occupy positions which are geometrically closer to the negatively charged points on the clay lamina, the base-exchange sites. These ionic migrations provide experimental evidence for Pauling's electrostatic valence principle which was applied to clays by Hendricks.[9] The principle states that ionic systems are statistically neutral on the smallest possible scale. In freshly prepared hydrogen–bentonite, the hydrogen ions have not reached their most stable positions. Due to the small ionic radius of the hydrated proton it is conceivable that if enough base-exchange sites are available, most of the hydrogen ions in the hydrogen–clay system would eventually migrate into the laminar structure of these layer silicates, whereupon they would attain their maximum covalency.

(8) F. Kayser, J. M. Bloch and G. Gommery, *Bull. soc. chim. France*, 462 (1951).

(9) S. B. Hendricks, *Ind. Eng. Chem.*, **37**, 625 (1945).

Department of Chemistry
Kansas State College
Manhattan, Kansas

3β,17α-Dihydroxynorcholanic Acid Lactone

By August I. Ryer and William H. Gebert

Received March 17, 1952

Recently we reported the isolation of a new by-product of the oxidation of sitosterol acetate