Essentially similar results were obtained in another experiment.

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE
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Effect of Wetting on the Nitrogen Adsorption–Desorption Isotherm of a Silica Aerogel

BY MARVIN F. L. JOHNSON AND HERMAN E. RIES, JR.

A large pore, high area silica aerogel has been transformed without sintering into a small pore xerogel structure with negligible change in BET area.[1] The silica aerogel was prepared under the supervision of Dr. J. L. Gring of these Laboratories, using the methanol exchange method of Kistler.[2] The small pore xerogel type sample was obtained by immersing the aerogel in water (room temperature, 1.5 hours) followed by drying (110°, 12 hours; 593°, 2 hours).

The two nitrogen adsorption–desorption isotherms (Fig. 1) nearly coincide in the lower relative pressure region indicating similar BET areas: aerogel, 796 sq. m./g.; wetted aerogel (xerogel), 813 sq. m./g. BET plots are good straight lines in the 0.05 to 0.25 relative pressure range for both materials. The aerogel adsorption at p_0 is six times that of the small pore system whose structure is a xerogel type. Pore volumes are thus, respectively, 3.90 cc./g. and 0.66 cc./g. Average pore radius (or platelet separation) calculations[3] from pore volume and BET area show a reduction from 98 to 16 Å. by the treatment. These average radii are in qualitative agreement with Kelvin radii calculated from the steepest portions of the desorption curves; that of the aerogel is also in agreement with electron microscope observations. The desorption isotherms, furthermore, demonstrate narrow pore size distributions for both materials.

Kistler, Fischer and Freeman have suggested a three-dimensional network of needles or filaments as the probable structure of silica aerogels and presumably of xerogels.[4] According to this picture and the adsorption data, drying a wetted aerogel causes shrinkage or the closer packing of the needle-like fibers whose combined surfaces comprise the total catalyst area. Additional observations qualitatively supporting this picture of the structure are: the very low bulk density of the aerogel, 0.14 g./cc.; the increase in density to 0.49 g./cc. on treatment; the extreme fragility of the aerogel compared to the xerogel. Furthermore, the optical microscope shows uniformity in the xerogel structure in agreement with the narrow distribution of pore size.

The above observations indicate that reexposure of an aerogel to water effects a contraction with no significant change in the surface area. Presumably, the same surface tension forces are operative as those which cause any hydrogel to contract during drying to the xerogel stage. The data appear to support the proposed picture of gel structure.[4] The results also support the applicability of the BET area method to small pore structures since it is generally accepted for large pore systems.

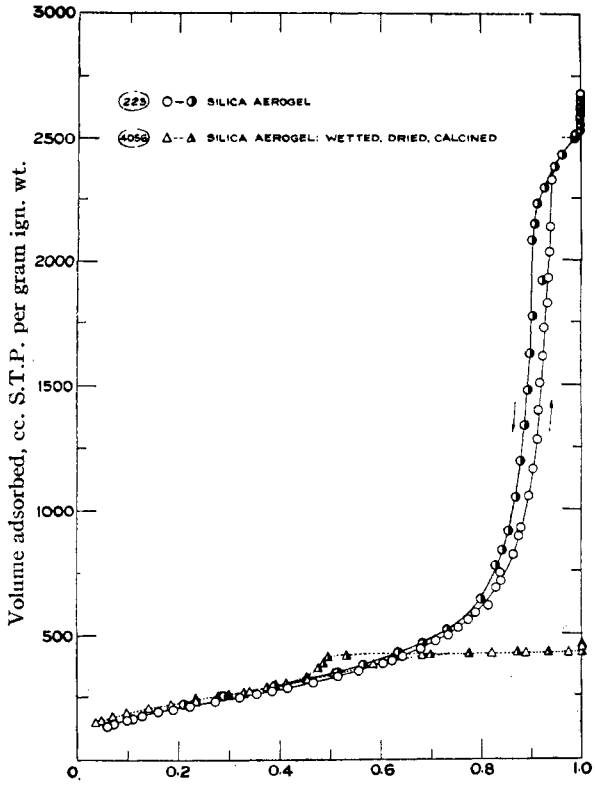

Fig. 1.—Effect of wetting on the nitrogen adsorption–desorption isotherm of a silica aerogel.

RESEARCH AND DEVELOPMENT DEPARTMENT
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Inhibition of Trypsin by Cholesteryl Malonic Acids and by *i*-Cholesteryl Acetic Acid

BY EMIL KAISER AND ROBERT HUBATA

The trypsin inhibiting effects of crystalline proteins prepared from pancreas,[1] from soybean meal,[2] and from egg white[3] have been reported. Salts of fatty acids,[4] salts of citric acid,[5] carbonyl

(1) Brunauer, Emmett and Teller, THIS JOURNAL, **60**, 309 (1938).
(2) Kistler, *J. Phys. Chem.*, **36**, 52 (1932).
(3) Ries, Johnson and Melik, *J. Phys. Colloid Chem.*, **53**, 638 (1949).
(4) Kistler, Fischer and Freeman, THIS JOURNAL, **65**, 1909 (1943).

(1) (a) Northrop, Kunitz and Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 1948; (b) Kazal, Spicer and Brahinsky, THIS JOURNAL, **70**, 3034 (1948).
(2) (a) Ham and Sanstedt, *J. Biol. Chem.*, **154**, 505 (1944); (b) Bowman, *Proc. Soc. Exp. Biol.*, **57**, 139 (1944); (c) Kunitz, *J. Gen. Physiol.*, **30**, 219 (1947).
(3) Balls and Swensen, *J. Biol. Chem.*, **106**, 409 (1934).
(4) Peck, THIS JOURNAL, **64**, 487 (1942).
(5) Pamfil and Maxim, *Klin. Woch.*, **17**, 1651 (1938).

group reagents[6] and several other substances[7] are, also, known to inhibit the activity of trypsin. In this report the trypsin inhibiting effect of salts of steroid carboxylic acids obtained from the condensation of cholesteryl *p*-toluenesulfonate with sodium malonic ester[8] is described.

The steroid acid inhibitors did not react stoichiometrically with trypsin as shown by Fig. 1. Similar to the action of crude trypsin inhibitors[9] an equilibrium reaction occurred. The 3-cholesterylmalonic acid was stronger in trypsin inhibiting activity than the *i*-cholesterylacetic acid, which, in turn, was a more effective trypsin inhibitor than the *i*-cholesterylmalonic acid.

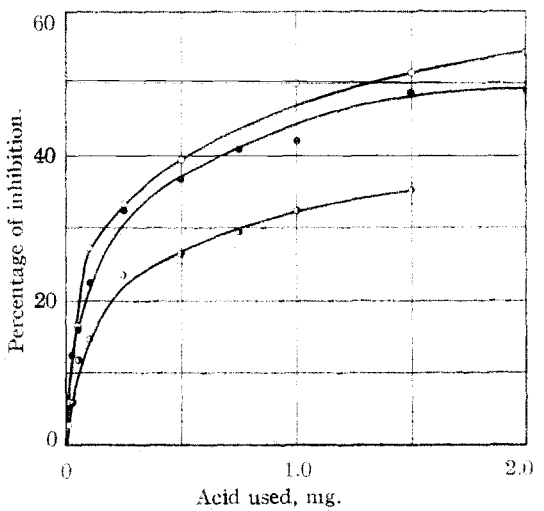

Fig. 1.—Showing the relationship between the percentage of trypsin inhibition and the amount of steroid acid used; one ml. of a solution containing 0.1 mg. of crystalline trypsin was inhibited with varying amounts of acids: O, 3-cholesterylmalonic acid; ●, *i*-cholesterylacetic acid; ◑, *i*-cholesterylmalonic acid.

Variation of the substrate concentration (hemoglobin was used as the substrate) did not affect the inhibition (Table I). Here, again, the results are in agreement with the findings for crude pancreas and soybean inhibitors[9] and indicate that the action of the inhibitors is non-competitive with the substrate.

Decrease of the trypsin concentration led to an increase in the inhibition (Table II). Crude pancreas and soybean inhibitors behave similarly.

TABLE I

INHIBITION OF CRYSTALLINE TRYPSIN; HEMOGLOBIN VARIED, TRYPSIN CONSTANT, INHIBITOR CONSTANT

Crystalline trypsin 0.1 mg., inhibitor acid 1.0 mg.

Hemoglobin substrate, ml.	Inhibition, % 3-Cholesteryl-malonic acid	*i*-Cholesteryl-malonic acid	*i*-Cholesteryl-acetic acid
5	49	28	38
4	47	30	39
3	50	24	37
2.5	49	25	34

TABLE II

INHIBITION OF CRYSTALLINE TRYPSIN; HEMOGLOBIN CONSTANT, INHIBITOR CONSTANT, TRYPSIN VARIED

Hemoglobin substrate 5 ml., inhibitor acid 1.0 mg.

Crystalline trypsin, mg.	Inhibition, % 3-Cholesteryl-malonic acid	*i*-Cholesteryl-malonic acid	*i*-Cholesteryl-acetic acid
0.1	47	27	43
.05	52	31	47
.025	71	41	67

A decrease of the inhibition of trypsin activity caused by fatty acids was observed when the fatty acids were precipitated as calcium salts.[4] When the cholesterylmalonic acids were precipitated with calcium acetate from trypsin-cholesterylmalonic acid mixtures, the inhibition increased (Table III). The calcium precipitation had no effect on the inhibition of trypsin by *i*-cholesterylacetic acid.

TABLE III

PRECIPITATION OF TRYPSIN-INHIBITOR MIXTURES WITH CALCIUM ACETATE

Mixture pptd. with $CaAc_2$, cryst. trypsin with	Inhibition % related to pptn. After	Before
3-Cholesterylmalonic acid	71	50
i-Cholesterylmalonic acid	44	26
i-Cholesterylacetic acid	40	40

These findings indicate that the steroid acids combine with trypsin through one of their carboxylic acid groups. In the case of 3-cholesterylmalonic acid and *i*-cholesterylmalonic acid an additional carboxylic acid group of the steroid acid is free to combine with calcium and to bring about a precipitation of the trypsin-inhibitor complex. In the case of the *i*-cholesterylacetic acid there is no remaining free carboxylic acid group in the steroid acid molecule after the trypsin-inhibitor complex is formed and, therefore, no precipitation occurs with calcium salts.

Not all the malonic acids nor all the steroid acids were found to inhibit the activity of trypsin. Malonic acid, ethylmalonic acid, cholic acid, dehydrocholic acid and adipic acid had no inhibiting effect on trypsin.

Experimental

The trypsin activity was determined according to the hemoglobin substrate method of Anson.[10] Crystalline trypsin of Armour and Company was used.

The steroid acids were suspended in water and 0.1 *N* sodium hydroxide added until the acids dissolved. The solutions were adjusted to *p*H 7.6 with dilute acetic acid and then diluted to the required concentrations. These concentrations varied from 0.01 to 2.50 mg. of steroid acid per ml. The solutions of 3-cholesterylmalonic acid, *i*-

(6) Schales, Suthon, Roux, Lloyd and Schales, *Arch. Biochem.*, **19**, 119 (1948).

(7) Grob, *J. Gen. Physiol.*, **29**, 249 (1946).

(8) (a) Kaiser and Svarz, THIS JOURNAL, **67**, 1309 (1945); (b) Svarz and Kaiser, *ibid.*, **69**, 847 (1947); (c) Kaiser and Svarz, *ibid.*, **71**, 517 (1949).

(9) (a) Northrop, *J. Gen. Physiol.*, **2**, 471 (1920); (b) Borchers, Ackerson and Sanstedt, *Arch. Biochem.*, **12**, 367 (1947).

(10) Anson, *J. Gen. Physiol.*, **22**, 79 (1938).

cholesterylmalonic acid and *i*-cholesterylacetic acid were slightly turbid at *p*H 7.6, but no precipitation occurred. Two other steroid acids, the 3-cholesterylacetic acid and the Δ^4-cholestene-6-acetic acid precipitated when the *p*H of their solutions was lowered to *p*H 7.6 and, therefore, were not used in the inhibition studies.

One ml. of a trypsin solution containing 0.1 mg. of crystalline trypsin and one ml. of the steroid acid solutions were mixed. Five ml. of the hemoglobin substrate solution[10] was added and the mixture digested. The volume of the digestion mixture was 1 ml. larger than the volumes used by Anson.[10] The volume of the blanks, also, was increased by one ml. The intensity of the color developed with the phenol reagent was read on a Coleman spectrophotometer at 650 mμ. The galvanometer readings of the blanks were not changed when the inhibitor acids were omitted.

A solution of 0.0008 milliequivalent of tyrosine was used as a reference standard. The color values of digestion products were calculated and the $10^4 \times (TU)^{HEM}$ read from the standard curve.[10] The difference between the units found in crystalline trypsin and the units found in crystalline trypsin-inhibitor mixture was the inhibited trypsin units. The percentage of inhibition was calculated from the inhibited trypsin units and the units found in crystalline trypsin.

The following series of determinations were made:

FIG. 1.—The concentrations of crystalline trypsin and hemoglobin were kept constant and the concentration of the inhibitor acids varied.

TABLE I.—The concentration of the hemoglobin solution was varied, the trypsin and inhibitor concentrations were kept constant.[9]

TABLE II.—The concentration of trypsin was varied and the concentration of hemoglobin and inhibitor was kept constant.

TABLE III.—The trypsin activity of a mixture of 0.1 mg. of crystalline trypsin with 1 mg. of 3-cholesterylmalonic acid, respectively, with 1 mg. of *i*-cholesterylmalonic acid or with 1 mg. of *i*-cholesterylacetic acid, was determined. Then 1 mg. of calcium acetate was added to these mixtures and the trypsin activity assayed again.

CHEMICAL RESEARCH AND DEVELOPMENT DEPARTMENT
ARMOUR AND COMPANY
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Association Phenomena in Some Organophosphorus Compounds

BY GENNADY M. KOSOLAPOFF AND J. STEPHEN POWELL

The recent investigations of dialkyl phosphites, $(RO)_2POH$, by means of the parachor[1] and the Raman spectra,[2] appear to indicate that these substances exist in the form of dimers, or possibly larger aggregates, which are held together probably by hydrogen bonds which involve the singular oxygen atom of each molecule. Since the structure of the POH portion of the molecule in these esters is common among the organophosphorus compounds with the phosphorus atom in a lower oxidation state, including such compounds as phosphinous acids, R_2POH, phosphonous acids, RPO_2H_2, and the mono-esters of the latter class, it was of interest to attempt a verification of such aggregation by other means. Among the most direct methods is the determination of molecular weights by cryoscopic means.

(1) Arbuzov and Vinogradova, *Izvest. Akad. Nauk S. S. S. R., otdel khim. nauk*, No. 6, 617 (1947).

(2) Arbuzov, Batuev and Vinogradova, *Doklady Akad. Nauk S. S. S. R.*, **54**, 603 (1946).

Determination of molecular weights, in benzene solution, of typical dialkyl phosphite and monoalkyl phosphonite failed to show any significant departure from the strictly monomolecular structure. However, the molecular weight of a typical phosphonous acid, namely, benzenephosphonous acid, was shown to be, within the experimental error limits, that of a trimeric aggregate. It appears that the presence of a true hydroxyl is essential for the display of such aggregation among the phosphorus derivatives and that the frequently postulated tautomerically formed POH in substances analogous to the dialkyl phosphites is, apparently, devoid of hydrogen bonding capacity, at least in solution in benzene. The trimeric aggregation of the phosphonous acid indicates the possibility of a cyclic trimer, which may be used to explain the long-known thermal disproportionation of these acids into phosphines and phosphonic acids, *via* the over-all scheme

$$3RPO_2H_2 \longrightarrow RPH_2 + 2RP(O)(OH)_2$$

This reaction therefore may proceed by the way of rearrangement of the protons and of the oxygen atoms within the trimeric cycle, in such a way as to produce the maximum concentration of oxygen atoms at two of the three phosphorus atoms. This phenomenon is rather common in this class of substances, for many of its classical reactions, such as the Arbuzov–Michaelis isomerization, involve the approximation to the highest possible number of semi-polarly linked oxygen atoms at the central phosphorus atom.

The effects of the variation of the substituent radicals upon the extent of association in these substances is being studied further.

Experimental

Diethyl phosphite showed no significant deviation from the monomeric molecular weight in benzene solution in cryoscopic method. Calcd. for $(EtO)_2POH$: mol. wt., 138. Found: mol. wt., 141, 141, 143.

Monobutyl benzenephosphonite gave similar results. Calcd. for $C_6H_5P(O)(OC_4H_9)H$: mol. wt., 198. Found: mol. wt., 200, 201, 200.

Benzenephosphonous acid, however, gave consistently trimeric molecular weights. Calcd. for $(C_6H_5PO_2H_2)_3$: mol. wt., 426. Found: mol. wt., 406, 399.

The preparation of this acid deserves some description. Phenyldichlorophosphine yields rather unsatisfactory specimens of this acid upon conventional hydrolysis with water; the product invariably possesses the repulsive odor of phenylphosphine. However, when advantage is taken of the ready cleavage of esters of trivalent phosphorus derivatives by hydrogen halides, a very convenient preparation of the acid may be had. The dichlorophosphine is added, without external cooling, dropwise with stirring into five volumes of absolute ethanol and the hot solution is diluted with two volumes of water, boiled for five to ten minutes, and evaporated under an infrared lamp to incipient crystallization. Addition of two or three volumes of benzene and the removal of the residual water and alcohol by the azeotrope distillation, followed by concentration of the benzene solution to incipient crystallization, yield the pure benzenephosphonous acid in the form of shiny colorless platelets, which melt sharply at 86°, and which possess no detectable odor of the phosphine. Since the product has a higher melting point than the original preparations made many years ago by Michaelis (m. p. 70–71°), which were made by conventional aqueous hy-