Anal. Calcd. for $C_{15}H_{10}O_7$: C, 59.6; H, 3.3. Found: after drying at 170° *in vacuo*, C, 59.2; H, 3.8.

The pentaacetate was prepared and melted at 200° (cor.). The mixed melting point with a known sample of the pentaacetate of quercetin was 200° (cor.). Sando gives 194 to 196° for the melting point of the pentaacetate of quercetin.[1] The absorption curves of the two samples of the pentaacetate in 95% ethanol were practically identical. The absorption maxima were at 2530 and 2990 A. with extinction coefficients (*E*, g. per liter, 1 cm.) of 40.0 and 34.5, respectively. Quercetin was regenerated from the pentaacetate preparations. The absorption curves of the regenerated quercetin preparations in 95% ethanol were practically identical. The maxima were at 2570 and 3750 Å. with extinction coefficients of 67.0 and 75.5, respectively, calculated on the dry basis. Grinbaumówna and Marchlewski give 2555 and 3755 Å. for the absorption maxima of quercetin.[2]

The micro-analyses were made by G. Warren Buckaloo and Lawrence E. Brown.

(1) C. E. Sando, *J. Biol. Chem.*, **117**, 45 (1937).

(2) R. Grinbaumówna and L. Marchlewski, *Biochem. Z.*, **290**, 261 (1937).

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NEW ORLEANS, LOUISIANA RECEIVED JULY 22, 1944

Observations on the Rare Earths. LII. The Preparation of Rare Earth Bromates from the Perchlorates

BY HOWARD E. KREMERS[1] AND THERALD MOELLER

In the preparation of rare earth bromates by metathetical reaction between rare earth sulfates and barium bromate,[2] significant quantities of rare earth materials are occluded by the precipitated barium sulfate. Furthermore, the method is complicated by the limited solubility of barium bromate.[2] Reactions between rare earth perchlorates and the more soluble potassium bromate overcome these objections, the precipitated potassium perchlorate showing less tendency to occlude rare earth salts than barium sulfate because of its somewhat greater solubility and consequent slower rate of precipitation. Bromates are also more readily prepared in this fashion than by treatment of rare earth oxides or hydroxides with bromic acid and are suited to fractional crystallization.

Experimental

Nearly neutral rare earth perchlorate solutions, prepared from yttrium group oxides by action with perchloric acid and containing the equivalent of 15 to 20% rare earth oxide, were treated with powdered potassium bromate and the resulting mixtures boiled for one hour. After being cooled to 15°, the suspensions were filtered and the residues washed with saturated potassium perchlorate solution until the washings were rare earth-free. These precipitates generally contained about 0.1% rare earth calculated as oxide, and never more than 0.5%.

Each filtrate was systematically fractionally crystallized to six fractions after fifteen crystallizations. Analyses of these fractions by standard methods showed the most insoluble fractions to consist of potassium bromate with traces of potassium perchlorate, the middle fractions to consist of rare earth bromates with traces of potassium bromate, and the most soluble fractions to consist of rare earth bromates with traces of rare earth perchlorates.

During the course of the fractionation, small amounts of potassium perchlorate and basic rare earth bromates precipitated. The latter never amounted to more than 1% of the total fraction, and such precipitations did not prove objectionable.

The preparation of rare earth bromates from perchlorates is more convenient and rapid than the preparation involving barium bromate, but the removal of by-products is not as complete. Since potassium bromate and perchlorate rapidly concentrate in the most insoluble fractions while remaining traces of perchlorate are carried through to the most soluble fractions, fractional crystallization is not impaired. Avoidance of loss of rare earth material in the initial precipitation constitutes the chief recommendation for the method.

(1) Present address, Lindsay Light and Chemical Company, West Chicago, Illinois.

(2) James, THIS JOURNAL, **30**, 182 (1908).

NOYES CHEMICAL LABORATORY
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URBANA, ILLINOIS RECEIVED JULY 10, 1944

Sterols from Peruvian Guano

BY JOHN KRUEGER

The sterol present in comparatively large amounts in Peruvian guano and provisionally called "guanosterol" or "guanosterine"[1] is really cholesterol as shown by the m. p. and m. m. p. of both the sterol and its acetate. Marker[2] has shown that the sterol present in largest amount in chicken feces is sitosterol. The cholesterol present in guano reflects the diet of the marine birds which produce the deposits.

Procedure.—Three pounds of Peruvian guano was stirred with 4 liters of ethanol at 40–50° for several hours, and the mixture then allowed to stand overnight. The residue was filtered and the filtrate was evaporated. The residue from the evaporation was refluxed with excess alcoholic sodium hydroxide, diluted with water, then extracted into ether. The pale tan sterol obtained by evaporation of the washed ether solution was recrystallized from 30 cc. of ethanol to yield 4.0 g. of cholesterol of m. p. 142° which showed no depression in m. m. p. when mixed with cholesterol. The acetate, prepared in the usual way, melted at 113° and showed no depression in m. m. p. when mixed with cholesteryl acetate.

(1) del Aguila, *Bol. soc. Quim. Peru*, **4**, 199–200 (1938) (*C. A.*, **33**, 2270 (1939)).

(2) Marker and Shabica, THIS JOURNAL, **62**, 2523 (1940).

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Electrophoresis of Rat Sera[1]

BY CHOH HAO LI

In the last few years, many investigators[2] have studied the electrophoresis of animal and human sera, but apparently no studies with rat sera have

(1) Aided by grants from the University of California Research Board, the Josiah Macy, Jr., Foundation, New York City, and General Mills, Inc., Minneapolis, Minnesota.

(2) Referred to, for example, by H. Svenson, *J. Biol. Chem.*, **139**, 805 (1941).

Fig. 1.

Fig. 2.

Fig. 1.—Normal rat serum, diluted 1:1. Exposures taken on the descending boundary.

Fig. 2.—Hypophysectomized rat serum, diluted 1:1. Exposures taken on the descending boundary.

been reported using the optical techniques of Longsworth[3] or Svensson-Philpot.[4] Recently Levin[5] found a decrease of serum-albumin concentration in rats following hypophysectomy; his conclusions were based on the results obtained by chemical analysis. It appeared, therefore, of some interest to study the electrophoretic behavior of sera of normal and hypophysectomized rats.

Male rats forty days of age of the Long–Evans strain were used. The post-operative period following hypophysectomy was two weeks and the completeness of the operation was ascertained carefully at autopsy by examination of the sella turcica. The animals were maintained on the usual diet of this Laboratory *ad libitum*. Blood was taken directly from the heart and was usually obtained from 3 or more rats for one sample. No significant difference in protein concentration was observed in normal and hypophysectomized rat sera as found by Levin.[5]

Electrophoresis experiments were carried out in the apparatus of Tiselius[6] with the optical arrangement of Longsworth.[3] The sera were usually diluted with an equal volume of the buffer and dialyzed against 2 liters of the same buffer for at least three days. The buffer was prepared from sodium diethylbarbiturate with *p*H 8.5 and ionic strength 0.10. In most cases the period of electrolysis was one hundred twenty minutes with a potential gradient of about 6 volts per cm. Since under these circumstances the false boundary was not fully separated from the slowly moving real component the concentration–distribution measurements were obtained from the descending boundary only.

(3) L. G. Longsworth, THIS JOURNAL, **61**, 529 (1939).

(4) J. St. L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).

(5) L. Levin and J. H. Leathem, *Am. J. Physiol.*, **136**, 306 (1942).

(6) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

A typical electrophoresis pattern of normal rat serum is shown in Fig. 1. There are not less than five components. According to the conventional designation they may be called, in the order of decreasing mobilities, albumin, x, α-, β-, and γ-globulins. The x component[7] is also found in mice serum.[9] The sharp and high β-globulin peak has been found in all normal rat sera thus far examined. As in the rabbit serum, the albumin concentration is about 75% of the total protein which is in contrast with the sera obtained from horse, cow and swine.[2] This means that the albumin–globulin ratio is about 3.0.

In the sera of hypophysectomized rats, there are no indications of the x component. This appears to be a characteristic[10] of such sera in contrast with sera obtained from normal rats. As shown in Fig. 2, it is evident that the globulin concentration is higher than in the normal rat. The albumin–globulin ratio from five samples of hypophysectomized rat sera[11] averaged 1.33.

Table I summarizes the albumin and globulin concentration distribution data in the normal and hypophysectomized rat sera. Since the separation of the globulin components was imperfect, no attempts were made to calculate the percentage composition of each globulin. Due to the high concentration of protein, no accurate mobilities could be obtained; the mobility deter-

TABLE I

PER CENT. COMPOSITION OF ALBUMIN AND GLOBULIN IN NORMAL AND HYPOPHYSECTOMIZED RAT SERA

No. of rats	Body weight at bleeding	Relative concentration Albumin	Globulin	Albumin/ globulin
		Normal Rats		
5	140	72.0	28.0	2.58
4	138	70.8	29.2	2.42
3	141	70.4	29.6	2.38
5	138	79.2	20.8	3.80
				Mean 2.80
		Hypophysectomized Rats		
5	124	53.5	46.5	1.15
4	122	61.5	38.5	1.60
5	125	56.5	43.5	1.30
3	125	55.2	45.8	1.21
4	124	58.4	41.6	1.40
				Mean 1.33

(7) Some authors (ref. 8) have denoted the x component as α_1-globulin.

(8) See, for example, L. G. Longsworth, *Chem. Rev.*, **30**, 323 (1942).

(9) J. Bourdillon and E. H. Lennette, *J. Expt. Med.*, **72**, 11 (1940).

(10) In a private communication, D. H. Moore has not been able to find this difference between normal and hypophysectomized rat sera in Levin's samples. The discrepancy between their results and ours may depend on the dilution of sera used.

(11) L. Levin has kindly sent us a sample of hypophysectomized rat serum. The albumin–globulin ratio is found to be 1.5. Levin has obtained a ratio of 1.2 from the sample using the salt fractionation method. It may be added that we were not able to find the x component with this sample.

mination is therefore purposely omitted. However, the mobilities were used for identification of the components.

It will be noted that the albumin–globulin ratio is distinctly lower in the hypophysectomized rat serum in comparison with normal serum. The percentage lowering of this ratio is about 53.0. This is somewhat higher than that found by Levin,[5] who obtained a percentage lowering of 46.0.

Addendum.—While this note was in the hands of the Editors, an article by Moore, *et al.*,[12] appeared in which they found that the normal rat serum lacks the α-globulin. It may be noted in Fig. 1 that the appearance of this component is evident. It is possible that the sera they used are too dilute to escape the detection of a small concentration of the α-globulin component.

(12) D. H. Moore, L. Levin and J. H. Leathem, *J. Biol. Chem.*, **153**, 349 (1944).

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BERKELEY, CALIF. RECEIVED MAY 22, 1944

The Determination of Water in Formic Acid

BY J. MITCHELL, JR., AND WALTER HAWKINS

In a previous publication from this Laboratory[1] the Karl Fischer reagent was not recommended for the determination of water in the presence of formic acid, presumably because of dehydration of the acid. Later studies on this system have indicated that under normal conditions this interference is not appreciable, amounting to only a fraction of a per cent. in high concentrations of formic acid.

Experimental

Aqueous solutions of the acid were prepared by adding various amounts of water to Eastman Kodak Company formic acid.. Weighed samples were analyzed for water by direct titration with Karl Fischer reagent and for free acid by titration with standard alkali. Results are summarized in the following table.

THE DETERMINATION OF WATER IN FORMIC ACID

Water, wt. %		Acid, wt. %		Total, wt. %
89.85	± 0.05	10.22	± 0.01	100.07
70.30	0.10	29.76	0.02	100.06
26.1	0.1	74.0	0.2	100.1
14.80	0.00	85.20	0.00	100.00
1.55	0.05	98.80	0.02	100.35

(1) Smith, Bryant and Mitchell, THIS JOURNAL, **61**, 2407 (1939).

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The van der Waals Constant "a" from C_p/C_v Measurements

BY R. E. RUNDLE

By an improved resonance method Clark and Katz[1] have succeeded in obtaining accurate measurements of γ, (C_p/C_v), as a function of pressure for a number of gases. They find experimentally that for simple gases the variation of γ with pressure is linear, and they show that this is the expected behavior of a gas whose equation of state is $PV = RT + BP$. It is also interesting to note that a linear dependence of γ on pressure is to be expected for a van der Waals gas at moderate pressures, and that from the slope of the curve, γ *vs.* P, the van der Waals constant a can be determined.

For a substance whose properties are a function of P and T only[2]

$$C_p - C_v = T\left(\frac{\partial V}{\partial T}\right)_P\left(\frac{\partial P}{\partial T}\right)_V \qquad (1)$$

For a mole of van der Waals gas

$$(P + a/V^2)(V - b) = RT \qquad (2)$$

$$\left(\frac{\partial P}{\partial T}\right)_V = \frac{R}{V - b} \qquad (3)$$

$$\left(\frac{\partial V}{\partial T}\right)_P = \frac{R}{P - a/V^2 + 2ab/V^3} \qquad (4)$$

Substituting (2), (3) and (4) in (1), and ignoring $2ab/V^3$ with respect to a/V^2

$$C_p - C_v = R\frac{P + a/V^2}{P - a/V^2} \qquad (5)$$

In the term a/V^2 it suffices to use the molal volume from the perfect gas equation. Then

$$C_p - C_v = R\frac{(RT)^2 + aP}{(RT)^2 - aP}, \text{ or} \qquad (6)$$

$$\gamma = \left(\frac{R}{C_v} + 1\right) + \frac{R}{C_v}\frac{2aP}{(RT)^2} + \ldots \qquad (7)$$

where the coefficients of higher powers of P are small, so that the extra terms may be ignored at moderate pressures. It is to be noted that C_v is independent of pressure for a van der Waals gas, so that $C_v = R/(\gamma_0 - 1)$, and the dependence of γ on pressure is linear.

If terms in higher powers of P are necessary, the term in b cannot be ignored. In this case

$$\gamma = 1 + R/C_v\left[1 + \frac{2aP}{(RT)^2} + \frac{2a}{(RT)^3}(a/RT - b)P^2 + \ldots\right] \qquad (8)$$

For certain gases, Clark and Katz find that γ at constant T must be expressed in terms of an equation of the form

$$\gamma = \gamma_0 + C_1P + C_2P^2 + \ldots$$

but the correlation with equation (8) is not good. Apparently the approximation of a real gas by the van der Waals equation is not sufficient to make the coefficient of P^2 in (8) significant. It appears, however, that the coefficient of P can be used to calculate a, just as in equation (7).

Equation (7) has been applied to data of Clark and Katz[1,3] to obtain a for a number of gases. In the table these values of a are compared with

(1) Clark and Katz, *Can. J. Research*, **18A**, 23 (1940); **21A**, 1 (1943).

(2) Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., New York, N. Y., 1923, p. 136.

(3) Clark and Katz, *Can. J. Research*, **19A**, 111 (1941).