NOTES

The Isolation of Vitamin K as a Choleic Acid

By H. J. Almquist and A. A. Klose

We wish to report the preparation of vitamin K choleic acid, a molecular compound of the vitamin with deoxycholic acid. This compound may prove useful in isolating the pure vitamin, especially since the vitamin appears inert to reagents that attack specific groups [*J. Biol. Chem.*, 125, 681 (1938)].

Concentrates prepared by methods already noted [This Journal, 61, 532 (1939)] were dissolved in warm ethanol and an amount of deoxycholic acid equal to five times the weight of the concentrate was added. The solution was evaporated to dryness using gentle heat and a stream of inert gas. The solid was then dissolved in approximately 2 cc. of methanol per 100 mg., and 0.5 cc. of water was added. After thorough mixing and cooling at 0° for several hours, the precipitate was filtered off and dried. In some cases the product was recrystallized by the same procedure. When working with cruder concentrates such as those which we obtain after molecular distillation, it was found preferable to add water to the methanol solution to the point of distinct turbidity only, and to reject the first crop of crystals,

which was usually inactive. Fractional crystallization was continued by concentrating the solution or by adding more water to it. In Table I are given representative data on various fractions obtained and their vitamin K activities.

As shown in the case of preparations F and D which were made from less pure concentrates, first an inactive fraction with a melting point at approximately 195° has been obtained. A second fraction melting at approximately 185° has proved quite active. Third and fourth fractions and the residual solids in the mother liquors, all of much lower melting points than the second fraction, invariably have shown no activity. The active fraction has been successfully decomposed by extraction with xylene and by molecular distillation.

From the purest concentrates yet prepared, we have repeatedly obtained a vitamin K choleic acid with a melting point of 186–187°. No other fraction, except deoxycholic acid itself, could be isolated. Activity of these preparations has also been tested by oral administration to deficient chicks. Restoration of normal . clotting time occurred within twenty-four hours, indicating high activity. An ether extract of a sample of vitamin K choleic acid was tested and found active in the same way.

From preliminary titrations of the choleic acid, we estimate that it contains about 10% vitamin K. Thus some of the most active preparations fed at a level of 75 mg. per kilogram have furnished 7.5 mg. of the vitamin, with resulting low blood clotting times which approximate the blood clotting times from feeding the purified concentrate.

In interpreting these values, it must be recalled that the assay method we now use [*Biochem. J.*, 32, 1897 (1938)] is more rigorous than our former methods, inasmuch as chicks are depleted of vitamin K for one week before use on assay. A comparison of blood clotting time results must take into account the assay methods and the standards employed.

The vitamin K choleic acid is a crystalline substance with a slight yellow color. When liberated from the choleic acid and from traces of solvent and deoxycholic acid, the vitamin has reap-

TABLE I
RESULTS ON VITAMIN K CHOLEIC ACIDS AND RELATED PREPARATIONS

Preparation	M. p., °C.	Level fed per kg. of diet, mg.	Av. blood clotting time, minutes	Number of chicks
F 1	195–196	100	>30	5
F 2	182–184	100	4.3	7
D 1	194–195	125	>17	4
D 2	185–186	125	3.1	4
M 1	182–184	75	4.0	9
M 1. xylene extract of 75 mg.	(oil)	≃75	4.5	9
M 1. solids in mother liquor	170–172	200	>30	8
H 1	186–187	....	...	..
H 1, molecular distillate	(oil)	7.5	4.3	8
H 1, distillation residue		100	>30	5
E 1 (from purified concentrate)	186–187	....	...	..
O 1 (from purified concentrate)	186–187	75	3.7	5
Reference standard solution[a]	(oil)	16 cc.	4.0	20
Purified concentrate	(oil)	5	7.4	7

[a] Standard solution representing 1 g. of dried alfalfa per cc.

peared as a viscous, slightly pigmented oil. It bears no resemblance in physical properties to a crystalline product with a melting point of 69° claimed to be pure vitamin K by Doisy and co-workers [*Science*, **88**, 243 (1938)].

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Relation between Compressibility and Solubility of Metals in Acids

BY SOFRONIO BALCE

If we apply to metals the compressibility formula for compounds as given in an earlier paper,[1] the equation becomes

$$B_{calcd.} = \frac{V_a}{5.6 \times 10^{12} Z}$$

where $B_{calcd.}$ is the calculated compressibility, 5.6×10^{12} is a universal constant relating physical

TABLE I

SHOWING THE DIFFERENCES BETWEEN METALS THAT DISPLACE HYDROGEN FROM ACIDS AND THOSE THAT DO NOT

Metal	Z	Compressibility[a] in cc./atm. $B_{calcd.}$	$B_{obsd.}$	$\frac{B_{obsd.} - B_{calcd.}}{B_{calcd.}}$
Cs	1	12.9×10^{-6}	62×10^{-6}	3.81
Li	1	2.35	9.1	2.87
Rb	1	10.5	40.5	2.86
K	1	8.54	32.1	2.76
Na	1	4.3	15.8	2.67
Sr	2	3.05	8.1[b]	1.65
Ca	2	2.29	5.8	1.53
Mg	2	1.21	2.9	1.4
Al	3	0.61	1.34	1.2
Zn	2	.86	1.45	0.68
Cr	3	.45	0.73[c]	.64
Fe	3	.43	.654	.52
Cd	2	1.17	1.72	.47
Co	3	0.405	0.557	.375
Ni	3	.40	.542	.364
Sn	2	1.47	1.9	.29
Pb	2	1.65	2	.23
Cu	1	1.29	0.756	− .41
Ag	1	1.85	1.02	− .45
Pt	2	0.82	0.328	− .60
Au	1	1.845	.552	− .66
Ir	2	0.778	.244	− .89

[a] The figures on compressibility are from the "International Critical Tables."

[b] P. W. Bridgman, *Proc. Am. Acad.*, **70**, 285–317 (1935).

[c] T. W. Richards, "The Compressibilities of the Elements and Their Periodic Relations," Carnegie Institution of Washington, 1907.

(1) S. Balce, *Philip. J. Sci.*, **60**, 251–254 (1936) [*Chem. Zentr.*, **108**, II, 2113 (1937); British *C. A.*, A, I, 176 (1937); *C. A.*, **31**, 2881 (1937)].

properties of substances to shrinkage in volume per unit volume incident to compound formation, Z is the valence of the metal elements, and V_a, the atomic volume.

This calculated compressibility, however, does not check with observed values. But when the $B_{calcd.}$ calculated compressibility is less than the $B_{obsd.}$ observed value, the metal may be said to be comparatively soft and is subject to solvent action by non-oxidizing acids; and when the calculated compressibility is greater than the observed, the metal does not displace the hydrogen.

As may be noted in the accompanying table, the order in which the metals occur in the electromotive series can be approximated by dividing the deviation, $\Delta B = B_{calcd.} - B_{obsd.}$ by $B_{calcd.}$. The units of compressibility in the table are changed to cc./atmosphere.

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Note on the Solubility of Strontium Chromate

BY T. W. DAVIS AND J. E. RICCI

In connection with an attempted study of the solubility of strontium chromate in dioxane-water mixtures as solvents, which had to be abandoned because of the extreme slowness with which equilibrium is approached, some observations were made on the solubility of this salt in water.

The figure uniformly given for the solubility of strontium chromate at room temperature is the determination of Fresenius,[1] 0.12 g. in 100 g. of solution at 15°, which was a confirmation of work by Meschtschersky.[2]

The only values for other temperatures are those of Reichard[3]: namely, 0.465% at 10°, 1.000% at 20°, 2.417% at 50° and 3.000% at 100°. The last figure for 100°, is the one quoted in the "Handbook of Chemistry and Physics,"[4] in its current editions. These incredible figures are evidently the basis for the Noyes procedure for the qualitative analysis of the alkaline earths, in which one is cautioned against much washing of the strontium chromate precipitate which is then redissolved by passing hot water through the filter paper.

(1) Fresenius, *Z. anal. Chem.*, **29**, 418 (1890).
(2) Meschtschersky, *ibid.*, **21**, 399 (1882).
(3) Reichard, *Chem. Ztg.*, **27**, 877 (1903).
(4) Chemical Rubber Publishing Co., Cleveland, Ohio.