[CONTRIBUTION FROM THE DEPARTMENT FOR DISEASES OF THE CHEST, JEFFERSON HOSPITAL]

Some Salts of Levulinic Acid[1]

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Introduction

The therapeutic use of organic salts of metals depends considerably on the organic part of the molecule to which the metal is attached. This part affects the solubility of the salt, the stability and the *P*H of its solutions and the reaction of the drug on normal tissues, sometimes causing excessive irritation and necrosis and increasing in general the toxic action of the metal attached. Careful selection of the organic vehicle, the carrier of the metal in the therapeutically used salt, appears, therefore, to be very important, particularly when the salt is to be introduced by way of intramuscular or intravenous injection.

Calcium salts of different organic and inorganic acids are at present used therapeutically in various conditions, including tuberculosis. The calcium therapy of tuberculosis is based on the theory that the disease is accompanied by demineralization in general, and in particular by the loss of calcium in the blood and the tissues. Numerous attempts have been made to increase the amount of calcium in blood and stimulate calcification of tubercles by the use of calcium salts.

In the course of our study of calcium metabolism in tuberculosis,[2] we were unable to maintain a measurably high calcium content in blood by feeding the patients even large therapeutic doses of calcium salts. Intravenous injection of calcium was then attempted, and, for this purpose, it was found necessary to prepare a neutral salt of high calcium content—a salt easily soluble and stable in water solution and having low toxicity and no irritating effects. Of the various compounds tested in our laboratory, calcium levulinate proved to be the most suitable for intravenous injections. It has obvious advantages over calcium gluconate; it contains 40% more calcium, is much more soluble in water and its 30% solution remains uncrystallized in ampules for an indefinite length of time. Its toxicity was thoroughly tested by us on animals and its extensive trial on tuberculous patients showed no untoward reactions.

The calcium salt of levulinic acid described by Grote and Tollens[3] may be prepared directly from the levulinic acid, but a less costly procedure is to isolate the calcium salt directly from the mixture obtained by acid hydrolysis of sugar.

As the low toxicity of levulinic acid in its salts was well established by previous investigators[4] as well as by our own experiments on animals

(1) A part of the expense for this investigation was defrayed by the C. Mahlon Kline Fund.

(2) Gordon, Kough and Proskouriakoff, *J. Lab. Clin. Med.*, **18**, 507 (1933).

(3) Grote and Tollens, *Ann.*, **175**, 181 (1875).

(4) Weintraud, *Arch. expt. Path. Pharmakol.*, **34**, 367 (1894).

and men[5] it seemed to be desirable to prepare and try the levulinates of some other metals, the inorganic salts of which were tested and were shown to have some therapeutical value.[6]

The magnesium, cadmium, manganese and nickel levulinates were prepared directly from levulinic acid and a study of their toxicity and chemotherapeutical value is under way.

Experimental Part

Preparation of Calcium Levulinate.—On hydrolysis of sugar with hydrochloric acid by the method of Sah and Ma[7] or its modification suggested by Thomas and Schuette[8] a dark liquid containing some humus material was obtained. This was filtered and the hydrochloric acid was distilled off at atmospheric pressure. The volume of the liquid and its constitution vary according to the method and the material used for the hydrolysis.

To the cooled dark liquid thus obtained was added 5 volumes of water, using mechanical stirring during the addition. The black tarry precipitate was removed by filtration, whereupon an almost clear yellow liquid was obtained. This was shaken with charcoal (Norit), and, on filtering, resulted in a perfectly colorless clear liquid. The minimum amount of charcoal can be found, if desirable, by preliminary small test-tube experiments.

The clear, colorless solution was warmed almost to boiling and treated with small portions of calcium carbonate while being stirred, until the evolution of carbon dioxide ceased and the excess of calcium carbonate added remained undissolved. The solution was then allowed to stand overnight in a cool place.

It was then filtered and evaporated on the water-bath until crystallization began. On cooling, the crystals were filtered off and washed with alcohol to remove the slight yellow color.

To the mother liquor an equal volume of alcohol was added, whereupon an additional amount of a well-crystallized calcium salt of levulinic acid was precipitated on standing.

(The other salts of levulinic acid can also be prepared by this method when it is desirable to avoid the purification of the acid.)

The sample of the salt was dried in the oven at 130° for twenty-four hours and analyzed.

Anal. Calcd. for $(C_5H_7O_3)_2Ca$: Ca, 14.83. Found: Ca, 14.82, 14.76.

Magnesium, cadmium, manganese and nickel salts of levulinic acid were prepared by treating the boiling 10% solution of levulinic acid in water with a slight excess of corresponding carbonate adding it with stirring in small portions. After being allowed to stand in a cool place for about twenty-four hours the solutions were filtered and evaporated on the water-bath.

Magnesium Levulinate.—The solution which contained magnesium levulinate was evaporated until it reached the consistency of thick paste. This was placed in a vacuum desiccator over sulfuric acid for about four days, when it turned into a solid, during which time it was occasionally stirred or crushed. It was then pulverized in a glass mortar and the powder was immediately placed in a vacuum desiccator for two more days. It was found to be very hygroscopic. The powder then was dissolved in a very

(5) Dr. Burgess Gordon and others, unpublished.

(6) Lumier and Chevrotier, *Bull. gen. Therap.*, **165**, 959 (1913); Walbum, *Z. Tuberk.*, **51**, 209 and 273; **53**, 292 (1929); Lunde, *ibid.*, **54**, 114 (1929).

(7) Sah and Ma, THIS JOURNAL, **52**, 4880 (1930).

(8) Thomas and Schuette, *ibid.*, **53**, 2324 (1931).

small volume of absolute methyl alcohol and the solution stirred into a large volume of anhydrous ether. The oily substance solidified on stirring. It was filtered off, washed with anhydrous ether and dried in a vacuum desiccator; white powder; quickly absorbs water from the air; easily soluble in water and methyl and ethyl alcohol; insoluble in ether; water solutions neutral to litmus.

Cadmium Levulinate.—The solution which contained cadmium levulinate was evaporated until it began to crystallize. After cooling it was placed in a vacuum desiccator over sulfuric acid for twenty-four hours. The resulting compact mass of crystals of very thin, hair-like structure was crushed, placed in a large glass mortar and triturated with acetone. The crystals were then filtered off with suction, washed with acetone and placed in a vacuum desiccator for another twenty-four hours. The trituration with acetone was then repeated, the precipitate filtered off, washed with anhydrous ether and dried in a desiccator. The cadmium salt can be purified by precipitation with anhydrous ether from its concentrated solution in absolute alcohol. The heavy oily substance first formed solidifies on stirring and scratching: colorless crystals, m. p. 145°, easily soluble in water and methyl and ethyl alcohols; insoluble in ether, acetone, ethyl acetate and petroleum ether. The salt was dried in the oven at 110° for twenty-four hours and cadmium was determined as cadmium sulfate.

Nickel Levulinate.—The solution which contained nickel levulinate was evaporated to dryness, the sticky paste being stirred at the end of the evaporation. The dry substance was ground in a mortar and put in a desiccator. It is a pale green powder easily soluble in water and methyl and ethyl alcohols. It can be precipitated from ethyl alcohol by adding a large volume of absolute ether. It is insoluble in acetone, ether, petroleum ether and ethyl acetate. It gradually decomposes on heating, but does not melt below 250°. The sample for analysis was dried in the oven at 100° and nickel was determined as nickel dimethylglyoxime.

Manganese Levulinate.—The solution which contained manganese levulinate was shaken with Norit and filtered. It was evaporated until it appeared as a thick sirup. It was then put into a vacuum desiccator over sulfuric acid for forty-eight hours. The crystalline mass was twice triturated with acetone in a mortar, filtered, thoroughly washed with acetone and finally with ether, and first dried in air, then placed in a vacuum desiccator: colorless crystals—long prisms, m. p. 114°, easily soluble in water and alcohol insoluble in acetone, petroleum ether and ether. The sample was dried *in vacuo* at 70° for forty-eight hours and the manganese was determined as manganese pyrophosphate.

ANALYSES

Formula:	$(C_5H_7O_3)_2Mg$	$(C_5H_7O_3)_2Cd$	$(C_5H_7O_3)_2Ni$	$(C_5H_7O_3)_2Mn$
Calculated:	Mg, 9.56	Cd, 32.82	Ni, 20.32	Mn, 19.28
Found:	Mg, 9.59	Cd, 32.80	Ni, 20.54	Mn, 19.22

Summary

1. A convenient method for the preparation of calcium salt of levulinic acid has been described.

2. Cadmium, magnesium, nickel and manganese salts of levulinic acid have been prepared for pharmacological study.

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