XIV. INVESTIGATIONS ON THE NATURE OF CALCIUM BOROGLUCONATE

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FOR some time before 1935, solutions of calcium gluconate were employed in the treatment of acute hypocalcaemia, notably milk fever in cows. Injection of this substance was, however, often attended by serious local reaction, due chiefly to the low solubility of the gluconate. Dryerre & Greig [1935] having confirmed the observation of Hayden [1934] that boric acid greatly increases the solubility of calcium gluconate, advocated the use of calcium "borogluconate" in preference to calcium gluconate in cases of milk fever. The "borogluconate" is now widely employed with satisfactory results.

The name calcium "borogluconate" was suggested by Dryerre & Greig for the substance formed on evaporation of an aqueous solution containing five parts by weight of calcium gluconate and one part of boric acid, these being the proportions used for injection. The authors observed that the substance differed from boric acid and from calcium gluconate in that it gave no green flame coloration and possessed a different crystal structure. It was also about thirty times as soluble as calcium gluconate at 15°.

Apart from these facts, nothing was known about the nature of this "borogluconate" and it was with a view to obtaining more definite knowledge of the substance that the present experiments were undertaken.

Some of the substance was prepared according to Dryerre & Greig, evaporation being carried out under reduced pressure at 40° and also over a boiling water bath: there appeared to be no difference between the two specimens in the course of the experiments. Titration experiments on the "borogluconate" so obtained showed that in aqueous solution all the boron was present as free boric acid: the compound, if it existed as such, therefore seems to be wholly split up into its components in aqueous solution. This was borne out by the fact that when an aqueous solution of the "borogluconate" was partly re-precipitated by the addition of ethyl alcohol, the precipitate and the solution contained entirely different proportions of boric acid.

The fact that the substance exists as a mixture of its constituents in aqueous solution, that it is almost insoluble in alcohol and other solvents and that proper melting points for gluconate and "borogluconate" cannot be obtained, raised the difficulty of devising a method to show whether, in the solid state, the "borogluconate" is an actual compound or merely a mixture. This obstacle was overcome by employing a distillation method, ethyl (or methyl) borate being volatile and easily distilled. If a mixture of calcium gluconate and boric acid is distilled with ethyl alcohol, all the boric acid is driven off as the ester. This was carried out with a mixture of the two substances in the proportions employed by Dryerre & Greig, and after distillation for a short time it was found that the residue contained no boric acid. On the other hand, if some "borogluconate" is prepared by evaporation of a solution containing the same proportions, and subjected to distillation with alcohol, the residue still retains all

of the boric acid. Obviously, then, the "borogluconate" is not merely a mixture, the boric acid being attached in some way to the gluconate molecule. A flame test experiment on the same lines bears this out. If a mixture of gluconate and boric acid is ignited with ethyl (or methyl) alcohol, a brilliant green coloration is imparted to the flame, whereas with the "borogluconate" no such coloration is obtained, unless some conc. H_2SO_4 is also present.

Considering the structure of the "borogluconate", the most likely possibility would seem to be the attachment, by loss of water, of the boric acid to a pair of *cis*-hydroxyl groups in the gluconate molecule. This is in accordance with the formulation first proposed by van't Hoff [1870] and more recently supported in numerous papers by Böeseken and his co-workers at Delft.

The proportions of calcium gluconate and boric acid employed by Dryerre & Greig do not coincide with those required for a compound of the gluconate with one or more molecules of boric acid. Accordingly experiments were carried out to determine, if possible, the amount of boric acid required to saturate the gluconate molecule.

A solution containing calcium gluconate and a large excess of boric acid was evaporated to dryness: the substance was then distilled for a considerable time with ethyl alcohol. The boric acid in the residue was then estimated and was found to correspond almost exactly with the amount required, on the van't Hoff formulation, for a compound of calcium gluconate with two molecules of boric acid. Repeated distillation of the residue failed to alter the proportion of boric acid. The compound obtained after distillation showed no green flame coloration with alcohol in the absence of conc. H_2SO_4 .

It may therefore be assumed that calcium borogluconate proper is a compound of one molecule calcium gluconate and two molecules boric acid and the following may be suggested as a probable formula:

EXPERIMENTAL

Estimation of boric acid. This was carried out in aqueous solution by titration with $N/20$ NaOH in the presence of excess glycerol, using phenolphthalein as indicator. The glycerol was added gradually during the titration until the pink colour no longer disappeared. Calcium gluconate, which was present in all the solutions under examination, was quite neutral to phenolphthalein as was the pure glycerol used. The gluconate was a "Sandoz" product.

Preparation of calcium "borogluconate". 6 g. calcium gluconate and 1.2 g. boric acid (H_3BO_3) were dissolved together in distilled water, warming to facilitate solution: the solution was then filtered and evaporated to dryness in a boiling water bath (or under reduced pressure).

Removal of free boric acid by distillation with alcohol. 1–2 g. of material were usually employed. The substance was put into a small (100 ml.) distilling flask with about 40 ml. absolute alcohol, and distilled on a water bath: more alcohol was added when the level became low and the distillation repeated. Two such

distillations were usually sufficient to remove all the boric acid. To estimate the remaining boric acid, the alcohol was filtered off from the residue, which was then dried in a vacuum desiccator, and a weighed quantity dissolved in water and titrated against standard alkali as described above.

Preparation of calcium borogluconate proper. This can be prepared by accurately weighing out the required proportions of calcium gluconate and boric acid (1 mol. : 2 mol.) but a more satisfactory method is to have present a slight excess of boric acid, and after evaporation of the solution to dryness, expel the excess by distillation with alcohol.

The calcium borogluconate is very soluble in water and in 20% solution has *pH* approximately 3.5, as compared with 3.7 for the "borogluconate" of Dryerre & Greig. An aqueous solution of a mixture of gluconate and boric acid (1 : 2 mol.) also has *pH* 3.5.

Summary of experimental results

Substance under examination	% H_3BO_3	% H_3BO_3
	Found	Calculated
Prepared "borogluconate"	16.53	16.67
Mixture of gluconate and boric acid (5:1)	16.50	16.67
Mixture after distillation with alcohol	0.00	—
"Borogluconate" after distillation with alcohol	16.80	—
Borogluconate proper, prepared with excess H_3BO_3 and distilled with alcohol	25.90	{ 13.60 (1 mol.) { 25.73 (2 mol.) { 36.00 (3 mol.)

It is to be noted that calcium gluconate and boric acid mutually increase each other's solubility in water. A 6% boric acid solution, for example, is quite stable at 15° in the presence of calcium gluconate, whilst a 25% gluconate solution is stable in the presence of 2% H_3BO_3 .

SUMMARY

Calcium borogluconate proper is a definite compound formed from one molecule of calcium gluconate and two molecules of boric acid by loss of water. In aqueous solution it behaves as a mixture of these two substances.

A formula is suggested; the substance may be called calcium diborogluconate. The "borogluconate" of Dryerre & Greig is a mixture of calcium mono- and di-borogluconates.

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