VIII. POLARIMETRIC DETERMINATION OF GLUCONIC ACID.

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THE specific rotation of malic, tartaric and other α -hydroxy-acids is markedly increased by the formation of complex compounds with uranium salts and with molybdates, tungstates, etc. Use is made of this phenomenon in the estimation of optically active malates and tartrates in plant material [Auerbach and Kruger, 1923]. The writer is not aware that this phenomenon has been made use of in the estimation of gluconic acid. It can, however, be conveniently applied to this purpose.

EXPERIMENTAL.

Calcium gluconate recrystallised from water was dried at 100° and a 0.1 *N* (2.15 %) solution was prepared. 5 ml. samples of this stock solution were mixed with 2.0, 5.0, 10.0 and 15.0 ml. respectively of saturated ammonium molybdate solution; 2.0 ml. of glacial acetic acid were added, and each sample was made up to 25 ml. with distilled water. The rotations of these solutions were measured at various time intervals after mixing the reagents.

The results of nine typical experiments are given in Table I. It is found that the rotation of the gluconate-molybdate mixture is at its maximum value as soon after mixing of the reagents as it is possible to take readings (about 5 minutes). The rotation decreases during the first two hours to a value which is maintained almost unchanged for some days. It will be noted from Table I that the rotation of the gluconate-molybdate mixture attains its maximum value when 5 ml. of saturated ammonium molybdate are present in 25 ml. of solution. Trebling the concentration of molybdate only brings about a 3 % decrease in rotation, and halving the molybdate concentration correspondingly brings about a reduction in rotation of about 10 %.

Table I.

	ml. saturated molybdate per 25 ml. of solution	$[\alpha]_D^{25} *$		
		1 hour	3 hours	24 hours
I	2.0	343°	314°	—
II	2.0	331	—	—
III	5.0	372	361	361°
IV	5.0	373	—	366
V	5.0	371	—	—
VI	10.0	384	—	—
VII	10.0	362	352	—
VIII	15.0	362	350	—
IX	15.0	347	—	—

* Calculated with respect to quantity of total gluconic acid.

On the basis of these and other similar results, it was decided to treat gluconic acid or gluconate solutions in the following standard manner. 8 ml. of the gluconate solution are mixed with 5 ml. of saturated ammonium molybdate and 2 ml. of glacial acetic acid, and the mixture is allowed to stand for at least 3 hours in darkness. The rotation is then measured. Under these conditions, as the figures in Table II show, the relationship between gluconate content and rotation is almost linear when the gluconic acid content is less than 1.5 % and deviates only slightly at concentrations up to 3 %. In general, plant material containing gluconates or gluconic acid also contains carbohydrate. The specific rotation of glucose is not directly affected by treatment with the molybdate reagent, but relatively concentrated solutions (5 % for example) are fairly rapidly oxidised by the molybdate mixture, which itself becomes dark blue in colour. The rotation of the solution increases probably owing to the formation of gluconic acid among the products of oxidation of the glucose, and polarisation of the darkly coloured solution becomes impossible.

In the presence of sugars therefore the standard procedure mentioned above is varied. A measured volume of the solution is boiled with sufficient excess of NaOH to neutralise the acid and convert any gluconic lactone into sodium gluconate; calcium chloride is added and the solution treated with alcohol to make the alcohol content 80 %. The precipitated calcium salt is filtered, washed and redissolved in water so as to give 8 ml. of solution, to which 5 ml. of molybdate and 2 ml. of glacial acetic acid are then added. After standing 3 hours in the dark the solution is polarised as before.

Table II gives the observed rotations in a 2 dm. tube, and also the specific rotations when solutions of various gluconic acid contents were used in the standard manner described. The quantity of gluconic acid recorded in the left-hand column of the table is the quantity present in the 8 ml. sample used which is made up to 15 ml. by addition of molybdate and acetic acid. Sodium light was used in all cases and the temperature throughout was 22°.

Table II.

Quantity of gluconic acid, mg. in 8 ml.	Observed rotation in 2 dm. tube	Specific rotation
19.6	0.96°	367°
39.2	1.93	369
78.4	3.83	366
117.6	6.10	389
156.8	8.00	381

Intermediate values have been obtained by interpolation. It has been shown that the presence of citric acid does not affect the value of the specific rotation of gluconic acid in a manner comparable with the effect on malate-molybdate mixtures. An 8 ml. sample containing 78.4 mg. gluconic acid and 192 mg. citric acid in standard molybdate mixture gave a rotation of 3.96° in a 2 dm. tube. The specific rotation is therefore 378°.

Table III.

Acid	Specific rotation in presence of molybdate	Specific rotation in presence of uranyl salts
<i>d</i> -Malic	- 720 to - 770°	+ 510 to + 525°
<i>l</i> -Malic	+ 720 to + 770	- 510 to - 525
<i>d</i> -Gluconic	+ 366 to + 390	About + 80

The gluconate-containing material so far investigated in this laboratory has not contained malic or other optically active acids. It should, however, be possible to deal polarimetrically with such mixtures relatively easily on account of the different magnitudes (and directions) of the enhancements of rotation produced by molybdates and uranyl salts, of which data are given in Table III.

SUMMARY.

1. The specific rotation of *d*-gluconic acid is enhanced about 40-fold by formation of complex salts with molybdates. The value of the enhanced rotation is to a marked extent unaffected by variation in concentration both of gluconic acid and molybdate.

2. The rotations of gluconic acid complexes with uranyl salts and molybdates are in the same direction, in contrast to the behaviour of malates; hence polarimetric analysis of mixtures should be relatively simple.

REFERENCE.

Auerbach and Kruger (1923). *Z. Unters. Nahr. Genussm.* **46**, 97, 179.