

*Citation:*

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previously as to the direct nitration capacity of aliphatic carbon compounds, by nitrating malonic acid and its esters, methylenetricarboxylic ester etc. from which it follows that the direct nitration capacity is caused by the adjacency of so-called negative groups of definite strength.

In this manner were also discovered the aliphatic nitramines and nitramides, by nitrating the amides wherein occurs also a negative group and it now appears that an aliphatic secondary amine (dimethylamine) may also undergo direct nitration when in the alkyl groups are present the group CN or CO<sub>2</sub>H, so distinctly negative groups.

I, therefore, put to myself the question whether the phenyl or nitrophenyl-group would also be able to give the same result as CN or CO<sub>2</sub>H. This, however, does not seem to be the case, for dibenzylamine HN(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sub>2</sub> yielded only dinitrodibenzylamine nitrate, but no nitramine on boiling with absolute nitric acid.

The ready nitration capacity of iminodiacetonitrile and of iminodiacetic acid and its derivatives is striking especially when we compare it with that of other substances as shown in the subjoined list.

CH <sub>3</sub> .NH.CH <sub>3</sub>	not	CN.CH <sub>2</sub> .NH.CH <sub>2</sub> .CN	readily
CH <sub>3</sub> .NH.CO.CH <sub>3</sub>	readily	CO <sub>2</sub> H.CH <sub>2</sub> .NH.CH <sub>2</sub> .CO <sub>2</sub> H	readily
CH <sub>3</sub> .CO.NH.COCH <sub>3</sub>	readily	CO <sub>2</sub> .CH <sub>3</sub> .CH <sub>2</sub> .NH.CH <sub>2</sub> .CO <sub>2</sub> .CH <sub>3</sub>	readily
CH <sub>3</sub> .NH.CO <sub>2</sub> CH <sub>3</sub>	readily	C <sub>6</sub> H <sub>4</sub> (NO <sub>2</sub> )CH <sub>2</sub> .NH.CH <sub>2</sub> .C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	not
CH <sub>3</sub> .CO.NH.CO <sub>2</sub> CH <sub>3</sub>	readily	C <sub>6</sub> H <sub>2</sub> (NO <sub>2</sub> ) <sub>3</sub> .NH.CH <sub>3</sub>	readily
CH <sub>3</sub> .CO <sub>2</sub> .NH.CO <sub>2</sub> CH <sub>3</sub>	not	C <sub>6</sub> H <sub>2</sub> (NO <sub>2</sub> ) <sub>3</sub> .NH.C <sub>6</sub> H <sub>2</sub> (NO <sub>2</sub> ) <sub>3</sub>	not

**Biochemistry.** — “*A biochemical method of preparation of l-Tartaric acid.*” By Prof. J. BÖESEKEN and Mr. H. J. WATERMAN.  
(Communicated by Prof. BEIJERINCK).

In our investigations on the assimilation of carbon nutriment by different kinds of mould it was found necessary to get some more information as to the manner in which the carbon was retained in the body of the plant either temporarily or permanently.

For this purpose one of us (H. J. W.) carried out a large number

of carbon determinations of the mould that formed on a definite organic compound used as exclusive carbon nutriment, while at the same time the amount of unattacked substance in the nutrient base was determined. The further particulars of this research will be communicated by him elsewhere, when the significance of the survey thus obtained of the course of the plastic equivalent or *assimilation quotient of the carbon*<sup>1)</sup> will also be explained: here we will call attention to some observations on the growth of *Aspergillus niger* and *Penicillium glaucum* on the tartaric acids.

*Aspergillus niger* grew hardly at all on *l*-tartaric acid, but very well on the *d*-acid, so that a 2% solution of the latter provided with the necessary inorganic nutriment was, after the lapse of six days used up by the mould. A 4% solution of uvic acid provided with 0.15%  $\text{NH}_4\text{NO}_3$ , 0.15%  $\text{KH}_2\text{PO}_4$  and 0.06%  $\text{MgSO}_4$  and inoculated in the usual manner with *Aspergillus niger* and cultivated at 33°—34° gave after six days a maximum *l*-rotation; this then began to decrease slowly, showing that the *l*-tartaric acid also gets consumed.

This maximum rotation observed with the saccharimeter of SCHMIDT and HAENSCH for white light in a 20 cm. tube, amounted to  $-1^{\circ}.0$  corresponding with a solution of about 1.2% *l*-tartaric acid.

As a 4% solution of uvic acid can only give at most a 1.88% solution of *l*-tartaric (on account of the disappearance of the *d*-acid and the water of crystallisation of the uvic acid) this maximum rotation corresponds with a yield of fully 60%.

In order to isolate the *l*-tartaric acid the liquid after removal of the film of mould, was precipitated with lead acetate. The precipitate, after being washed was decomposed with hydrogen sulphide and the filtrate evaporated.

From 4 grams of uvic acid was obtained 0.8989 gram of *l*-tartaric acid = 56%.

The acid crystallises readily.

0.100 gram consumed 8.6 cc. of baryta water of which 4.66 were equivalent to 0.100 gram of salicylic acid: Mol. Weight = 149.3 (calculated 150)

0.2719 gram was dissolved in 50 cc. Polarisation in a 40 cm. tube =  $-0^{\circ}.8$ , that is, for a solution in a 20 cm. tube =  $-1^{\circ}.5$ , while a 2% solution of pure *d*-acid gave  $+1^{\circ}.6$ .

As a part of the *l*-acid is consumed and as this is connected with

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<sup>1)</sup> Compare WATERMAN, Mutation with *Penicillium glaucum* etc. These Proc. 29 June 1912, p. 124.

the increase of the mould material, it is desirable not to subject large quantities of uvic acid to this operation all at once, but to distribute it into a number of small flasks (containing not more than 50 cc.). Operating in this manner we prepared from 40 grams of uvic acid, distributed into 20 flasks, nearly 9 grams of pure *l*-tartaric acid.

The question, which is more of a biological than of a chemical nature, how the *l*-tartaric acid is decomposed, could be answered by the determination of the above quoted assimilation quotient of the carbon, in that sense that it takes part, in the same degree as the *d*-acid, in the construction of the organism. This could not be proved in a direct manner, because the pure *l*-acid promoted the growth of the inoculating material too slowly, but it could be determined from the values obtained in the growth on uvic acid compared with that of *d*-tartaric acid.

The *l*-acid is evidently attacked only a little slower. If we mix *d*-acid with uvic acid this will not much affect the absolute consumption of the *l*-acid because the total amount of mould material formed in the same time will be approximately the same. As, however, we can only subject to the operation a solution not exceeding 6%, the quantity of *l*-tartaric acid is smaller from the commencement and the yield will have to be low. If, for instance, we mix 1½ gram of *d*-tartaric acid with ½ gram of uvic acid, practically no *l*-acid will be left when all the *d*-acid has disappeared.

These experiments with *Aspergillus* were carried out in conjunction with others, because we had noticed that *Penicillium glaucum*, which was used for many of our observations exhibited towards the tartaric acids a but little pronounced power of selection. This will be seen at once from the subjoined table.

The three acids behave nearly similar in regard to a same *Penicillium* culture; only during the first days the growth on the *l*-acid is somewhat less than on the two others.

It seems remarkable that, in consequence of the great concentration of the hydrogen ions, the retardation which in a 2% solution of *l*- and *d*-acid is very plainly perceptible after six days<sup>1)</sup> does not set in at all with the anti-acid. This is quite in harmony with the smaller dissociation constant of this acid<sup>2)</sup> owing to which, in a

1) When using larger flasks, the surface in regard to the capacity is as a rule more unfavourable than when small flasks are used, so that the aeration becomes insufficient.

2) BÖESEKEN and WATERMAN. These Proc. 30 March 1912, p. 1112.

3) BISCHOFF and WALDEN. Ber. D. Ch. G. 22, 1819 (1889).

TABLE I.

Development of *Penicillium glaucum* in 50 cc. tapwater, provided with 0.05 %  $\text{NH}_4\text{Cl}$ , 0.05 %  $\text{KH}_2\text{PO}_4$  and 0.02 %  $\text{Mg SO}_4$ .

$T = 21^\circ$ .

	added acid in mg.	Development in stated number of days.		
Nº.	d-tartaric acid	2	4	6
1	50	+	+>	++
2	100	+	+>	++
3	300	+	+>	++>
4	500	+	+	++<
5	1000	+	+	+
	l-tartaric acid			
6	50	+	+	++<
7	100	+	+	++<
8	300	+	+	++
9	500	?	+	++<
10	1000	?	+	+>
	Antitartaric acid <sup>1)</sup>			
11	50	+	+	++
12	100	+	+>	++>
13	300	+	+	++>
14	500	+	+	++
15	1000	+	+>	++

2 % solution thereof, the harmful concentration of the H-ions for *Penicillium* is not yet reached.

For the rest, these small differences in growth at lower concentrations are somewhat unexpected because the specific character of the two anti-podes has been determined by PASTEUR first of all with *Penicillium glaucum*. But it may be very well assumed that the

<sup>1)</sup> *Aspergillus niger* gives practically no growth on antitartaric acid.

organism used by us has been another form than that employed by PASTEUR in his classic experiment and the continued investigations of one of us <sup>1)</sup> (H. J. WATERMAN) have exactly demonstrated that the phenomena of growth are dependent in a high degree on the variation.

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**Chemistry.** — *“On a method for a more exact determination of the position of the hydroxyl groups in the polyoxycompounds”.*

(4<sup>th</sup> Communication on the configuration of the ring systems).<sup>2)</sup>

By Prof. J. BÖESEKEN. (Communicated by Prof. A. F. HOLLEMAN).

The investigations as to the action of the polycompounds on the conductivity of boric acid were started to furnish a contribution to our knowledge as to the situation of the carbon atoms, and the groups attached thereto, in benzene.

This object has been attained to a certain extent, but, in addition, the measurements have also taught us something about the position of the hydroxyl groups in the saturated polyvalent alcohols.

The influence of polyoxycompounds and boric acid on each other has been known for a long time.

So, for instance, the increase of the acidic properties of boric acid by means of glycerol was made use of in the titration of that acid and, reversely, the large increase in rotation exerted by boric acid on mannitol went to demonstrate that this polyatomic alcohol was indeed optically-active<sup>3)</sup>. These few empirical data were very considerably added to by G. MAGNANINI<sup>4)</sup>; at the same time an experimental foundation was given to the surmise that these phenomena might be due to the formation of compounds.

He demonstrated that mannitol strongly increased the electric conductivity power of boric acid and that, although to a less extent, this was also the case with oxy-acids such as tartaric acid, salicylic acid, lactic acid, glycerine acid, gallic acid, mandelic acid and glycollic acid. He thus proved the formation of complex ions, consequently of a chemical combination between the two components.

VAN 'T HOFF<sup>5)</sup>, on account of these investigations, was of opinion

<sup>1)</sup> H. J. WATERMAN. These Proc. 29 June 1912, p. 124.

<sup>2)</sup> Recueil **30**, 392; **31**, 80 and 86.

<sup>3)</sup> VIGNON. Ann. Chim. Phys. 5e S. II 433. (1874).

<sup>4)</sup> Gazz. chim. **20**, 428; **21**, II, 134, 215. Zeitschr. phys. Chem. **6**, 58.

<sup>5)</sup> Lagerung der Atome im Raume. 3e Ed. p. 90.