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TRANSACTIONS

Synthesis of Ascorbic Acid and Its Identity with Vitamin C.

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The author has already stated in his preceding papers⁽¹⁾ that ascorbic acid isolated from suprarenal glands as well as from many vegetables, such as "Daikon" juice (*Daikon*, *Rhapanus Sativus* L.), Japanese green tea, etc. is identical with vitamin C. The possibility is, however, not yet excluded that the antiscorbutic potency may be exerted by a trace of some unknown substance still adhering to the acid. So the author has prepared ascorbic acid synthetically and proved that it has the same potency as the natural product. The synthesis was carried out after T. Reichstein according to the following scheme:

The synthetical preparation was identical, not only in its physical and chemical properties, but also in its antiscorbutic power, with natural ascorbic acid, a daily dose of 0.8 mg *per os* being sufficient to cure the scurvy of guinea pigs.

EXPERIMENTAL

The author, in synthesizing ascorbic acid, followed as strictly as possible the directions described by T. Reichstein⁽²⁾, and the result so obtained in every step of the above mentioned series of reactions agreed substantially quite well with Reichstein, so far as the author has examined. For this reason, only a brief account will be given below for some necessary knowledge of the intermediate reaction products and of the last one which the author has obtained.

1-Sorbose: Sorbitol was first prepared from glucose by reduction with sodium amalgam⁽³⁾ and then converted into sorbose by biochemical oxidation⁽⁴⁾ with *bacterium xylium*^{*}, according to the directions recommended by Schlubach and Vorwerk⁽⁵⁾. Sorbose obtained in this way melted at 159—161°.

Monoacetone-1-sorbose (Fig. 1). After recrystallization from acetic ether, it melted at 92—93°.

Diacetone-1-sorbose (Fig. 2). After recrystallization from benzine (b p 60—80°), it melted at 77—78°.

5.546 mg subst.:	11.204 mg CO ₂ ,	3.925 mg H ₂ O	
	Found	C 55.10	H 7.92%
C ₁₂ H ₂₀ O ₆	Calc.	" 55.35	" 7.75%
0.0500 g subst. dissolved in water to 5 cc:	$\alpha_D^{20} = -0.19$, $c=1.00$, $l=1$ dm		
	$[\alpha]_D^{20} = -19^\circ$		

Diacetone-2-keto-1-gulonic acid. The potassium salt of this acid (Fig. 3) was dried over calcium chloride to constant weight at ordinary temperature and analyzed.

3.924 mg subst.:	6.500 mg CO ₂ ,	2.100 mg H ₂ O	
2.739 mg subst.:	0.739 mg K ₂ SO ₄		
	Found	C 45.18	H 5.99 K 12.11%
C ₁₂ H ₁₇ O ₇ K	Calc.	" 46.12	" 5.49 " 12.52%

2-Keto-1-gulonic acid (Figs. 4 and 5): The product obtained from diacetone-2-keto-1-gulonic acid was recrystallized from water. It melted at 165° with decomposition when heated rapidly in a bath, previously heated to 150°.

4.331 mg subst.:	5.820 mg CO ₂ ,	1.965 mg H ₂ O	
3.805 mg subst.:	5.155 mg CO ₂ ,	1.810 mg H ₂ O	
	Found	C 36.65; 36.95	H 5.08; 5.32%
C ₈ H ₁₀ O ₇	Calc.	" 37.22	" 5.16%
0.0500 g subst. dissolved in water to 5 cc:	$\alpha_D^{20} = -0.47$, $l=1$ dm, $c=1.00$		
	$[\alpha]_D^{20} = -47^\circ$		

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Methylester of 2-keto-l-gulonic (Fig. 6): This substance was prepared from 2-keto-*l*-gulonic acid and melted at 155—157°.

4.571 mg subst.:	6.480 mg CO ₂ ,	2.405 mg H ₂ O		
	Found	C	40.45	H 5.89%
C ₇ H ₁₂ O ₇	Calc.	"	40.36	" 5.82%

Ascorbic acid (Fig. 7): The crude product obtained from methylester of 2-keto-*l*-gulonic acid melted at 175—178°. After recrystallization from methyl alcohol-petroleum ether-ether, it melted at 185—187° with decomposition. When mixed with natural ascorbic acid having the same m p, it showed no depression.

3.890 mg subst.:	5.835 mg CO ₂ ,	1.625 mg H ₂ O		
	Found	C	40.91	H 4.67%
C ₆ H ₈ O ₆	Calc.	"	40.89	" 4.58%
9.195 mg subst. dissolved in 0.5040 g water: $\alpha_D^{20} = +0.48^\circ$ $d_{20}^{20} = 1.0076$				
$c = 1.805$, $l = 1$ dm, $[\alpha]_D^{20} = +26.59$				

The substance gives positive reactions with silver nitrate, dichlorophenol-indophenol and ferric chloride, but no reaction with Millon's reagent. It gives neither turbidity nor precipitate with bromine water. Another point of interest is that the synthetic ascorbic acid, like the natural one, does not give Molisch's reaction with α -naphthol and sulphuric acid. It may be here remarked that Szent-Györgyi's first preparation of ascorbic acid⁽⁶⁾ must have still contained some sugar-like or glucosidic impurity which showed Molisch's reaction.

Therapeutic Experiment with Synthetic Ascorbic Acid.

In order to ascertain whether or not the synthetic ascorbic acid is antiscorbutically effective, a therapeutic test was made with the crude preparation melting at 175—178°. The procedure used was just the same as described in the preceding paper⁽¹⁾. The following chart shows the result obtained.

Four test animals Nos. 122, 123, 124 and 125, at first fed only on the usual vitamin-C-deficient basal diet, suffered from scurvy, but, when given on and after the 11th day each a dose of 0.8 mg of the test substance *per os* daily, they restored their usual health and normal growth. After 52 days they remained completely healthy. They were then subjected to autopsy and revealed no symptoms of scurvy. Of 2 control animals, Nos. 126 and 127, one was fed on both the basal diet and sorbose, in order to confirm that sorbose, though prepared from sorbitol by a biochemical method, possesses no antiscorbutic effect, and the other was fed on the basal diet alone. They died on the 24th day or so, suffering from severe scurvy. In the autopsy, they showed marked symptoms of scurvy.

Chart—Growth-curves of guinea pigs fed on vitamin-C-deficient basal diet with or without synthetic ascorbic acid. Animals Nos. 122—125 took each a dose of 0.8 mg of the synthetic ascorbic acid preparation of mp $175-178^{\circ}$ per os daily. The control No. 125 took daily a dose of 1 mg of sorbose, No. 127 received no supplement. The lines denote: ----- basal diet alone, ——— basal diet and ascorbic acid, — · — · — basal diet and sorbose.

Fig. 1—Monoacetone-*l*-sorbose, recrystd. from acetic ether.

Fig. 2—Diacetone-*l*-sorbose, recrystd. from benzene.

Fig. 3—Potassium salt of Diacetone-2-keto-*L*-gulonic acid, recrystd. from absolute alcohol.

Fig. 4—2-Keto-*L*-gulonic acid, recrystd. from water.

Fig. 5—2-Keto-*L*-gulonic acid, recrystd. from water.

Fig. 6—Methylester of 2-keto-*L*-gulonic acid, recrystd. from methyl alcohol.

Fig. 7—Ascorbic acid (Vitamin C) (synthetic)
recrystd. from acetone dioxane.

The above results indicate that the synthetic ascorbic acid has the same potency as the natural acid.

Therefore, it can be concluded that ascorbic acid itself is vitamin C.

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