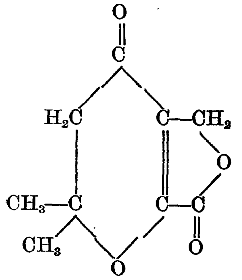
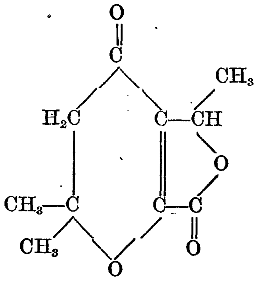
Two homologues, IV, m.p. 85° C., C found 59.48, 59.36, calc. 59.33, H found 5.82, 5.77, calc. 5.53, and V, m.p. 142° C., C found 60.99, calc. 61.23, H found 6.27, calc. 6.12, of compound II were also synthesized.

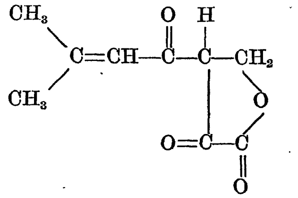


IV



V

IV was obtained by condensing ethyl oxalate with mesityl oxide in the presence of sodium methoxide and reacting the resulting sodium salt with formaldehyde to form VI, m.p. 150° C.



VI

This product gave a deep red color with $FeCl_3$ in aqueous alcoholic solution. VI was cyclicized to IV by heating it above its melting point, or by refluxing it in alcohol with piperidine as a catalyst, or by heating it with aqueous-alcoholic sulfuric acid. IV gave no color with $FeCl_3$. Molar quantities of sodium methoxide or diethylamine in alcohol reconverted IV to VI. V was prepared in a similar fashion to IV using acetaldehyde instead of formaldehyde.

Compounds II, IV and V behaved similarly to clavacin in that on treatment with dilute alkali and reacidification, they gave a deep red coloration with $FeCl_3$. This most probably is due to the opening of the pyrone ring, similar to the conversion of IV to VI, using sodium methoxide or diethylamine. Excess of alkali opens the lactone ring and leads to the formation of sodium oxalate and other degradation products.

IV is very stable towards dilute acids, even on boiling for a considerable length of time. When tested against *Staphylococcus aureus in vitro* compound II showed a slight bacteriostatic action in a dilution of 1:2000; IV, V and also III were inactive; VI had bacteriostatic activity in a dilution of 1:4000.

Katzman *et al.*[10] and Bergel *et al.*[9] have shown that clavacin on catalytic hydrogenation at atmospheric pressure takes up from 3–4 moles of hydrogen. We have found that compound IV takes up only 1 mole of hydrogen using palladium charcoal, Raney nickel or platinum oxide at 300 pounds pressure. The double bond in the pyrone ring was not reduced under these conditions, but a practically quantitative yield of anhydro-3-hydroxymethyl-5,6-dihydro-6,6 dimethyl-4-hydroxy-pyrone-carboxylic acid-2, m.p. 122° C., was obtained. This product was reconverted in good yields to IV by oxidation with chromic acid.

Our syntheses of compounds of type II and the study of their properties show that these compounds do not enter into the dynamic tautomerism suggested by Bergel *et al.*[9] for clavacin. The existence of the two isomeric forms, I and II, is comparable to the existence of the α- and β-forms of angelica lactone. Attempts to effect a rearrangement of compounds of type II to compounds of the clavacin type (I) have thus far been unsuccessful.

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THE EFFECT OF MACERATION OF FOODS UPON THEIR ASCORBIC ACID VALUES

THE methods of assay of foods for their ascorbic acid content, which have been recommended by the Committee on Vitamin Assay Methods of the National Cooperative Experimental Project on the Conservation of the Nutritive Value of Foods, stress the importance of correct sampling and the necessity of stopping the destructive action of the enzymes naturally present in certain plant foods by quickly extracting the ascorbic acid in a 3 per cent. meta-phosphoric acid solution. Although attention has been called to the fact that some of the ascorbic acid in foods may be present in the reversibly oxidized (also biologically active) form which is not measurable by the dye reduction method, the error from this source has not generally been considered serious.

Recently in this laboratory, however, the error under some conditions has been found to be of great magnitude. In a study of the vitamin A and C values of certain kinds of melons[1] we encountered considerable variation in the ascorbic acid content of different samples taken from the same melon. In an attempt to obtain more representative, homogeneous samples, the edible portions of whole cantaloupe were cut and 50-gm samples of the macerated material were taken for analysis. We were surprised to find that the ascorbic acid when analyzed by the usual method for the reduced form appeared to have been completely destroyed. When, however, H_2S was bubbled through a portion of the meta-phosphoric acid ex-

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[1] M. C. Smith, H. Farrankop and E. Caldwell, *Mimeo. Rept.* No. 67, Arizona Agricultural Experiment Station.

TABLE 1

REDUCED AND DEHYDROASCORBIC ACID IN CANTALOUPE BEFORE AND AFTER MACERATION*

	Ascorbic acid mg/100 gm		
	Reduced	Total	Per cent. dehydro
Before maceration	49	51	4
After maceration	0	49	100
After maceration and holding 4 hours	0	41	100

* 1 minute in Waring Blendor.

tract, following the method of Bessey,[2] it was found that almost all the ascorbic acid was still present but was in the reversibly oxidized form. The conversion was evidently catalyzed by the presence of ascorbic acid oxidases, for when the cantaloupes were steamed before they were macerated, the ascorbic acid remained in the reduced form.

The dehydroascorbic acid which had been so quickly formed when the melon cells were ruptured was evidently itself much more slowly changed to a biologically inactive substance. After holding the raw macerated material in which all the ascorbic acid was in the dehydro form for 4 and 24 hours, the loss in total ascorbic acid was only 16 and 35 per cent., respectively.

The possibility that conversion of reduced to dehydroascorbic acid may occur in raw vegetables when prepared for consumption, as quickly as it did in melons when macerated, suggested itself. As recently reported in SCIENCE,[3] "In many mess halls vegetables and fruits are finely minced." The losses in ascorbic acid which resulted in 30 minutes and 2 hours after mincing with steel or plastic knives or in the "Buffalo" chopper were reported. Although it was stated that "analyses were run by the method of Bessey,"[2] whether total ascorbic acid or the reduced form only was determined is not clear.

From our data, as shown in Table 2, it appeared that when apples, celery, cucumbers, peppers and radishes were chopped as is commonly done when they are served in salads, or when the juice was extracted as in Health Food Stores, a high percentage of the reduced ascorbic acid which had disappeared was still present in a biologically active form. Although some of the dehydroascorbic acid was destroyed on standing 4 or 24 hours, the losses were relatively small. When potatoes were boiled a loss in reduced ascorbic acid occurred as expected, but the action of ascorbic acid oxidase which catalyzes the conversion of the reduced to the dehydro form had obviously been inhibited. When ripe tomatoes were sliced, or their juices expressed, then ascorbic acid remained in the reduced form. The opposite was true for *green* tomatoes, for their ascorbic acid when juiced was all converted to the reversibly oxidized form and remained in this form after holding 4 hours.

TABLE 2

EFFECT OF PREPARATION OF RAW FOODS FOR CONSUMPTION ON THEIR VITAMIN C VALUES

Food	Method of preparation before sampling	Ascorbic acid/100 gm			
		Reduced		Total (Reduced and dehydro)	
		mg	Per cent. loss	mg	Per cent. loss
Apples	Uncut	3	...	5	...
	Chopped	0	100	5	0
	Juice	0	100	5	0
Celery	Uncut	8	...	12	...
	Chopped	5	38	12	0
	Juice	0	100	9	25
Cucumbers	Uncut	9	...	13	...
	Chopped	5	44	12	9
Peppers (green)	Uncut	70	...	77	...
	Chopped	38	46	74	4
	Juice	0	100	60	22
Potatoes	Uncut	30	...	32	...
	Chopped	3	90	28	13
	Boiled	21	30	24	25
	Boiled and mashed	10	67	14	55
Radishes	Uncut	28	...	31	...
	Chopped	19	32	29	10
Tomatoes (red)	Uncut	16	...	19	...
	Sliced	16	0	19	0
	Juice	16	0	19	0
(green)	Uncut	16	...	20	...
	Juice	0	100	20	0

Evidently the extent and rapidity of the conversion varied greatly with a food, its pH, enzymes present and the method of preparation for consumption.

The data in this paper thus indicate that the conversion of reduced ascorbic acid to the reversibly oxidized form proceeds so rapidly in some foods under many different conditions of sampling, holding, and preparation for consumption, and is frequently of such magnitude, that the usual method of assay of the reduced form *only* is an inadequate measure of the true vitamin C values if dehydroascorbic acid may be considered only slightly less active as an antiscorbutic than the reduced form.

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[2] O. A. Bessey, *Jour. Biol. Chem.*, 126: 771–784, 1938.

[3] C. M. McCay, M. Pijoan and H. R. Taubken, SCIENCE, 99: 454–5, 1944.

THE EXPERIMENTAL TRANSMISSION OF PINTA, MAL DEL PINTO OR CARATE TO THE RABBIT[1]

MOOSER, Varela and Vargas,[2] León-Blanco,[3] Briceño Rossi and Iriarte[4] and Lieberthal[5] tried, unsuccess-

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[1] From the Instituto de Enfermedades Tropicales y Parasitología "Carlos Finlay," University of Habana: