Isopropyl Oxalate, $[(CH_3)_2CH]_2C_2O_4$, colorless liquid boiling at 189°. Boiling point[1] previously reported is 190°.

Tertiary Alcohols.

Triphenyl carbinol, diethyl-phenyl carbinol and diphenyl-methyl carbinol were studied. In the first two cases no oxalates were obtained, the alcohols being recovered. With the third, however, a small yield of the oxalic acid ester was produced. This was separated from the unchanged carbinol by treatment with cold benzene in which it did not dissolve, then crystallized from hot benzene in colorless pyramids softening at 110° and decomposing gradually between 170–185° with the evolution of gas.

0.2056 g. subst.; 0.6043 g. CO_2; 0.1119 g. H_2O.
Calc. for $C_{30}H_{26}O_4$: C, 80.0; H, 5.77. Found: C, 80.14; H, 6.04.

[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, MINNESOTA AGRICULTURAL EXPERIMENT STATION.]

THE PRESERVATION OF PLANT JUICES FOR ANALYSIS OF SUGAR CONTENT.[2]

BY D. O. SPRIESTERSBACH.

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It is desirable at times to preserve samples for future analysis. This was the case recently at the Minnesota Experiment Station while sorghum investigations were in progress; however, certain difficulties are immediately met with when the samples consist of plant juices, *i. e.*, the extreme ease with which sugars are destroyed by microörganisms and the fact that the common disaccharides are rather easily hydrolyzed. The first difficulty can of course be overcome by the use of preservatives which either by virtue of their toxicity can kill microörganisms or are able to inhibit their growth. The second must be overcome by a means which counteracts the hydrolyzing agent, in this case principally acids. At the same time the substances employed must not in any way interfere with the proper determination of the composition of a juice. It is obvious that relatively few of the usual preserving agents meet the above conditions.

Accordingly, at the suggestion of Prof. R. M. West, a few common preservatives were tried in a preliminary way upon sorghum juice. Most of them immediately showed themselves to be unsatisfactory, the exceptions being mercuric iodide dissolved in potassium iodide solution, phenol, and toluene. The former was chosen on account of its high toxicity to microörganisms, and toluene was selected rather than phenol since it appeared to be equally efficient at the same time and was free of certain

[1] *Ann.*, 139, 229 (1866).

[2] Published with the approval of the Director as Paper No. 95, Journal Series, Minnesota Agricultural Experiment Station.

minor disadvantages of phenol during the determination of the sugar content.

Toluene was always added in sufficient quantity to saturate the juice and to leave an excess of a few cubic centimeters to gather in droplets at the surface. The mercuric iodide was at first made up in solution in such quantity that the required amount could be added with a pipet when collecting samples in the field. Table I shows the effect of this dilution in the two last samples. The errors introduced by this dilution were so

TABLE I.—SHOWING THE PRESERVATIVE ACTION OF MERCURIC POTASSIUM IODIDE AND TOLUENE UPON SORGHUM JUICE.

No.	Preservative.	Date analyzed.	Per cent. sucrose.	Per cent. reducing sugar.[1] R.	Per cent. reducing sugar[1] after inversion. R'.	R'—R.
3173	None........................	10/17/14	8.9	4.8	13.4	8.6
		22	3.1	7.6	10.4	2.8
		27	0.4	7.7	7.6	....
3179	Toluene.....................	10/17/14	9.0	4.8	13.5	8.7
		22	7.2	6.9	14.0	7.1
		27	4.6	9.2	13.8	4.6
		11/ 4/14	2.2	11.6	13.7	2.1
		13	0.4	13.2	13.2	....
		6/ 7/15	....	13.5	13.6	0.1
3180	0.05% mercuric potassium iodide	10/17/14	10.1	3.7	13.5	9.8
		22	9.7	4.2	13.8	9.6
		27	9.1	4.5	13.9	9.4
		11/ 4/14	8.7	5.0	13.9	8.9
		13	8.2	5.6	13.7	8.1
		6/ 7/15	....	12.0	13.7	1.7
3181	0.05% mercuric potassium iodide plus 1.8 grams sodium carbonate.................	10/17/14	10.6	3.1	13.3	10.2
		22	10.5	3.5	13.7	10.2
		27	10.0	3.5	13.7	10.2
		11/ 4/14	10.0	3.7	13.7	10.0
		13	9.7	3.9	13.6	9.7
		6/ 7/15	....	6.5	13.4	6.9
3182	0.1% mercuric potassium iodide	10/17/14	10.3	3.3	13.1	9.8
		22	10.0	3.9	13.7	9.8
		27	9.4	4.1	13.7	9.6
		11/ 4/14	9.1	4.6	13.7	9.1
		13	8.4	5.2	13.6	8.4
		6/ 7/15	....	12.0	13.7	1.7
3183	0.5% mercuric potassium iodide	10/17/14	10.0	3.0	12.7	9.7
		22	9.8	3.4	13.3	9.9
		27	9.2	3.7	13.0	9.3
		11/ 4/14	9.0	4.1	13.0	8.9
		13	8.5	4.5	12.9	8.4
		6/ 7/15	....	11.0	12.9	1.9

[1] Calculated as glucose.

great that in subsequent experiments the equivalent amount of mercuric chloride required was suspended in one or two cc. of water on a watch-glass, and crystals of potassium iodide added as needed to the point of just dissolving the last trace of mercuric iodide. The resulting small amount of solution was added to the juice, and rinsed down with it, so that the error due to a volume change was negligible. The samples were all kept at room temperature in stoppered bottles.

The results of the first series of analyses are shown in Table I. No initial analysis could be made upon the sample of juice as a whole, because it was taken at an out-of-town sorghum mill two days before reaching the laboratory but the first analysis of Sample No. 3181 may be taken for reference as the original composition. The preservatives were added as shown, and on comparison it was discovered that the sample containing both mercuric potassium iodide and sodium carbonate was the best preserved. The total sugar (calculated as dextrose) remains very constant for over 6 months, while the sucrose shows only a slight inversion. This demonstrates the possibility of inhibiting the growth, as well as the killing of microörganisms which attack sugars, by means of preservatives. The control sample rapidly deteriorated, as was to be expected, due chiefly to the action of various yeasts and acid-forming bacteria.

TABLE II.—SHOWING THE PRESERVATIVE ACTION OF TOLUENE AND MERCURIC POTASSIUM IODIDE UPON AN ALKALINE SOLUTION OF DILUTED SORGHUM SIRUP.

Sample No.	Description.	Date analyzed.	Per cent. sucrose.	Per cent. glucose.
A	Diluted sirups made slightly alkaline with sodium carbonate	6/15/15	8.6	4.3
		19	8.4	4.4
		26	8.4	4.2
		7/ 3/15	8.2	4.0
		10	Slimy	...
B	Same plus toluene	6/19/15	8.4	4.4
		26	8.4	4.4
		7/ 3/15	8.5	4.3
		10	8.5	4.4
		17	8.7	4.4
		9/20/15	8.5	4.3
C	Same plus 1 g. mercuric potassium iodide per l.	6/19/15	8.4	4.5
		26	8.3	4.4
		7/ 3/15	8.5	4.3
		10	8.5	4.3
		17	8.6	4.3
		9/20/15	Slimy	...

These results being so promising, it seemed advisable to repeat and confirm them. Inasmuch as the sorghum season was over so that no natural juice could be obtained, some sirups were diluted with water and treated as a juice. In this material a different bacterial flora was present prob-

TABLE III.—SHOWING THE PRESERVATIVE ACTION OF TOLUENE, MERCURIC POTASSIUM IODIDE AND MERCURIC NITRATE UPON BOILED AND UNBOILED SORGHUM JUICE MADE ALKALINE WITH SODIUM CARBONATE.[1]

Sample No.	Description.	Date analyzed.	Per cent. sucrose.	Per cent. glucose. R.	Per cent. reducing sugar[2] after inversion. R'.	Per cent. R' — R.
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
A	2 g. sodium carbonate and 5 cc. toluene added	20	11.8	5.8	17.9	12.1
		31	11.3	5.6	19.1	13.5
		11/29/16	11.2	5.6	17.6	12.0
		1/ 2/17	6.2	10.6	15.3	4.7
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
C	Boiled, cooled, made to volume and 2 g. of sodium carbonate plus 5 cc. toluene added	20	12.0	5.4	18.7	13.3
		31	11.7	5.4	18.7	13.3
		11/29/16	11.5	5.2	17.6	12.4
		1/ 2/17	5.8	10.7	16.0	5.3
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
B	2 g. of sodium carbonate plus 0.1% mercuric potassium iodide added	20	11.9	5.8	17.9	12.1
		31	11.7	6.3	18.3	12.0
		11/29/16	8.2	8.6	22.2[3]	13.6
		1/ 2/17	6.0	9.8	16.0	4.2
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
D	Boiled, cooled, made to volume and 2 g. of sodium carbonate plus 0.1% mercuric potassium iodide added	20	12.0	5.4	18.3	12.9
		31	11.4	5.2	17.9	12.7
		11/29/16	11.2	5.0	18.7	13.7
		1/ 2/17	10.0	6.0	13.8	7.8
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
E	1 g. of sodium carbonate added, kept cool, and 2% mercuric nitrate added on the fourth day thereafter	20	10.2	6.1	17.2	11.1
		31	10.2	6.0	17.2	11.2
		11/29/16	9.4	5.0	16.4	11.4
		1/ 2/17	9.4	6.2	15.3	9.1
O	Original juice	10/13/16	11.8	5.8	17.9	12.1
F	Boiled, cooled, 1 g. sodium carbonate added, made to volume, and kept cool; 2% mercuric nitrate added on fourth day thereafter	20	12.0	5.4	17.9	12.5
		31	11.6	5.3	17.9	12.6
		11/29/16	11.3	5.3	17.2	11.9
		1/ 2/17	11.2	5.3	14.9	9.6

[1] Analyzed by A. J. Wuertz, Graduate Assistant.

[2] Calculated as glucose.

[3] Obviously erroneous.

ably consisting largely of those organisms possessing capsules and which in some manner are able to resist boiling for a time. The results are shown in Table II.

Little inversion took place in the control sample due to the presence of sodium carbonate and the type of bacteria present. The total sugar remains very constant in the preserved samples. The fact that sucrose was apparently formed in small amounts, probably is due to errors in the methods of analysis. No further analyses could be made after the samples became slimy for they could not be filtered after adding the clarifying solution. Both preservatives were apparently equally good.

When sorghum juice was again available, another series of analyses was made. The results are shown in Table III. From these results mercuric potassium iodide apparently possesses greater preserving power than toluene, due probably to its greater toxicity and non-volatility. It has been noticed, however, that the mercury gradually becomes reduced and precipitates out, thereby decreasing its efficiency. The boiled solutions have as a rule shown themselves to be most easily preserved.

Some preliminary tests have indicated that mercuric nitrate may be even more effective as a preservative than is mercuric potassium iodide. The results of one series of analyses only are given in Table III. This shows that mercuric nitrate is a good preservative, especially in alkaline solution; moreover, it was added on the fourth day since the beginning of the series, the samples meanwhile being kept in the refrigerator. It is necessary to add larger quantities of the mercuric nitrate than of the mercuric potassium iodide since it causes a heavy precipitation which does not appear on the addition of mercuric potassium iodide. Further investigations of the value of mercuric nitrate as a preservative are desirable.

Summary.

The preservation of sweet juices for subsequent analysis presents two great difficulties, the prevention of (1) fermentation, and (2) hydrolysis. The preservative action of toluene and mercuric potassium iodide has been accordingly tested. The latter seems to possess the greater preserving action, and both show maximum effect in boiled samples which have been rendered slightly alkaline. The recommended procedure is to take a sample of definite volume, neutralize with a slight excess of calcium carbonate, boil for a few minutes, add 2 grams of anhydrous sodium carbonate per liter of juice, then add the preservative, cool to the original temperature, make up to the original volume taken and shake well. A too great excess of sodium carbonate destroys some of the reducing sugar. The density of the original juice may be taken with a Brix hydrometer, and the sample of preserved juice taken for analysis with a sucrose pipet to the original Brix value. In this way, the weight of the original juice is known as a basis for calculation, and the sugar content of the sample of

preserved juice will be the same as the original at the same temperature.

Preliminary experiments and one series of analyses indicate that mercuric nitrate may possess greater preserving action than mercuric potassium iodide.

St. Paul, Minn.

[Contribution from the Chemical Research Laboratory, The Upjohn Company.]

AN EXAMINATION OF THE LEAVES OF ADONIS VERNALIS.

By Frederick W. Heyl, Merrisl C. Hart and James M. Schmidt.

Received November 14, 1917.

Adonis vernalis L. is an anemonaceous plant of the family *Ranunculaceae*. It grows natively in Central Europe and in Asia, where it is known as the Bird's eye, Pheasant's eye or False Hellebore.

Extracts of the drug were first introduced into medicine in 1879 by Bubnow,[1] who employed them as a cardiac stimulant. Cervello,[2] interested by the favorable report of Bubnow, described a method for the preparation of an active digitalis like glucoside from *Adonis vernalis*, following the method employed in the manufacture of "Digitalin German" from Digitalis seeds. The plant was macerated for two days with 50% alcohol, and the extract after filtration was precipitated with lead subacetate. The concentrated filtrate was rendered ammoniacal and the glucoside was precipitated with tannic acid. The precipitate was washed with water and boiled with alcohol in presence of zinc oxide. After evaporation of the alcohol the residue was extracted with absolute alcohol and coloring matter was precipitated from the concentrated solution by the addition of ether. The filtrate from this contained his active glucoside principle which he named adonidin. The chemical work is entirely indefinite, but the pharmacology of this preparation was well worked up. Cervello found that "adonidin" was ten times as active as a preparation Digitoxin(?) used by Koppe[3] in bringing about heart rest in frogs. In contrast to this we find that dosage of adonidin (Merck) given as high as 0.03 g. which is about fifteen times the dose for the digitoxin and about equal to that given for the mixed glucosides from digitalis seeds.

Kramer[4] worked up a parcel of *Adonis aestivalis* by extracting with 96% alcohol and adding an equal volume of water to the extract. This mixture was shaken with petroleum ether, ether, and with chloroform. The chloroform extract yielded an active glucosidic principle which could be precipitated from concentrated alcoholic solutions by the addition of

[1] "Dissertation," St. Petersburg (1880).

[2] *Arch. exp. Path. Pharm.*, **15**, 235 (1882).

[3] R. Koppe, *Ibid.*, 3 (1879).

[4] *Arch. Pharm.*, **234**, 452 (1896).