are under way with known mixtures of viable and non-viable seeds for the purpose of establishing a color scale from which the approximate percentage of germination of a given sample of seed may be estimated by means of the peroxidase test.

Tests on the temperature at which the oxidase and the peroxidase reactions are destroyed show some variation with different species of seeds.

Conclusions.

1. From the results obtained, the writer is convinced that the peroxidase reaction can be made use of in seed-testing laboratories for detecting non-viable seed and for distinguishing between seed of high, medium and low viability.

2. That lettuce, alfalfa and soy-bean seeds are unique in that they contain both oxidases and peroxidases.

3. That the vital property of seeds is contained in a substance (presumably an oxygenase) which has the power to activate molecular oxygen, when exposed to the air, peroxidases being formed, and that when this power is lost the seed loses its power to germinate.

4. That the peroxidase reaction may be further turned to account in determining the rate at which seeds lose their viability.

LEXINGTON, KY.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF POMONA COLLEGE AND JOHNS HOPKINS UNIVERSITY.]

THE IDENTIFICATION OF PHENOLS. II.

BY JAMES A. LYMAN[1] AND E. EMMET REID.

Received January 21, 1920.

In a former article[2] it was shown that *p*-nitrobenzyl bromide reacts readily with alkaline solutions of phenols to give well characterized ethers which may be used for the identification of the phenols. It was thought desirable to extend this work to other phenols.

Materials and Methods.

The *p*-nitrobenzyl chloride was made by nitrating benzyl chloride and separating the *para* compound by crystallization. The *p*-nitrobenzyl bromide was made by brominating *p*-nitrotoluene.

Into a 100 cc. flask was measured 25 cc. of 0.2 *N* sodium alcoholate and a nearly equivalent amount of the phenol. Except in the case of the salicylic acid esters the mixture was left slightly alkaline to avoid contamination of the product with the phenol. The reagent, usually 1.0 g. in amount, was added and the solution heated one hour under a

[1] The experimental work except that on salicylic acid esters was done at Pomona College by J. A. L.

[2] THIS JOURNAL, 39, 304 (1917).

TABLE I.

	Solvent.		First crop.		Second crop.	
	Vol.	%	Wt.	M. p. °C.	Wt.	M. p. °C.
o-Nitrophenol, 1 g. chloride	32	75	0.87	123	0.33	71
	40	58	0.67	130	.....	...
	37	73	0.66	130	0.02	127
p-Nitrophenol, 1 g. chloride	30	80	0.81	70–165	0.35	70
	65	84	0.57	165	emul.	...
	..	..	0.54	187.7	emul.	...
	..	..	0.51	187.4	emul.	...
2,4-Dinitrophenol, 0.8 g. chloride	45	53	0.45	68–241	0.25	68
	50	95	0.04	248	0.28	72
o-Chlorophenol, 1 g. bromide	37	63	1.20	97.5	0.07	83
	19	80	1.16	99	0.03	83
	..	..	1.14	99.4	trace	...
	..	..	1.06	100.1	trace	...
	..	..	1.00	100	trace	...
p-Chlorophenol, 1 g. bromide	37	63	1.19	101.5	...	...
	19	80	1.14	101.5	0.02	101.5
	..	..	1.12	101.3	...	...
s-Tribromophenol, 0.5 g. bromide	25	95	0.44	163.5	emul.	...
	70	81	0.41	163.5	trace	...
Guaiacol, 1 g. chloride	25	95	1.08	63	emul.	...
	35	66	0.99	63.6	emul.	...
	35	66	0.87	63.6	emul.	...
α-Naphthol, 0.8 g. chloride	30	80	0.84	133	emul.	...
	45	84	0.63	140	emul.	...
	60	79	0.61	140	emul.	...
β-Naphthol, 0.8 g. chloride	33	83	0.68	106.7	emul.	...
	49	70	0.68	106.5	emul.	...
Salol, 1 g. bromide	30	80	0.90	77	emul.	...
	20	74	0.63	80.5	0.15	69
	14	68	0.55	84	0.04	74
	19	66	0.42	86	0.03	69
	15	63	0.36	87	0.03	69
	15	63	0.31	87	0.02	85
p-Cresyl salicylate, 1 g. bromide	25	95	0.89	128	oil	...
	27	95	0.68	139.5	emul.	...
	40	72	0.63	142.5	...	...
	40	72	0.61	142.5	emul.	...
m-Cresyl salicylate, 1 g. bromide	40	72	0.81	oily	emul.	...
	19	80	0.39	95–100	emul.	...
	14	70	0.29	115	emul.	...
	..	..	0.27	118	emul.	...
	..	..	0.36	118	emul.	...

reflux condenser after which more or less water was added, the amount being judged by the readiness of the product to separate out on cooling. The crystals filtered off were called "First Crop" and those obtained by adding a large volume of water "Second Crop." The first crop was recrystallized till the melting point became constant.

Results.

The results are given in tabular form, the first line representing the original preparation and succeeding lines the recrystallizations of the product. Under "Solvent" are given the volume and strength of the alcohol from which the crystals were obtained.

Picric acid, carvacrol, *m*-aminophenol and *p*-cresyl salicylate were tried but did not give satisfactory results.

Salicylic Acid Esters.

It has been shown by many chemists[1] that when an ester of one alcohol is dissolved in another alcohol, the second alcohol replaces the first, till equilibrium is reached. Normally this reaction is extremely slow but becomes enormously rapid in the presence of sodium alcoholate, as has been shown by many investigators. An example of this has been given by Reid.[2] From the work of Anderson and Pierce[3] phenyl acetate does not seem to undergo this reaction, or at least not rapidly. The results given above with salol and with the *ortho* and *meta* cresyl salicylates agree with this, showing as they do, that the ethyl alcohol used as solvent did not replace the phenol or cresols in the salicylates, even when excess alkali was present.

p-Nitrobenzyl derivatives were prepared from methyl salicylate exactly as above, using a slight excess of sodium ethyl alcoholate and crystalline derivatives were readily obtained in excellent yields and worked up as usual. The one from methyl salicylate melted, crude, at 119° and, on recrystallization, at 123.8° and 124°. That from ethyl salicylate melted, crude, at 123.5° and, on recrystallization at 124.5° and 124.5°, showing it to be very pure from the start, while the derivative prepared starting with the methyl ester melted low at first. The practical identity of the melting points suggested that the methyl salicylate had been almost entirely transformed into the ethyl ester and that both products were the *p*-nitrobenzoyl derivative of ethyl salicylate, $p\text{-}NO_2C_6H_4CH_2O.C_6H_4.COOC_2H_5$. This appeared to offer an interesting case of ester transformation and these derivatives were further studied from that point of view. The derivatives of these esters and of *n*-butyl salicylate

[1] For a number of references and further discussions see Pardee and Reid, *J. Ind. Eng. Chem.*, **12**, 129 (1920).

[2] *Am. Chem. J.*, **45**, 506 (1911).

[3] *J. Phys. Chem.*, **22**, 49 (1918).

were prepared, using the corresponding alcohol as solvent in each case. The reagent was 1.0 g. of bromide in all of the preparations that follow.

TABLE II.

	Solvent.		First crop.		Second crop.	
	Vol.	%.	Wt.	M. p. °C.	Wt	M. p. °C.
Methyl salicylate in MeOH.....	30	83	1.05	128.2	oil	...
	35	100	0.89	128.2	0.11	127.5
Ethyl salicylate in EtOH.....	30	80	1.02	122–3	emul.	...
	18	87	0.93	124.2	0.06	95–8
	15	95	0.84	125	0.07	110
Butyl salicylate in BuOH.....	15	..	1.12	90.3	oil	...
	15	95	1.02	91–2	0.05	86.7
	15	MeOH	0.78	91–2	0.15	89
	18	85	0.68	92	0.06	90

In the last case, the first and third recrystallizations were from ethyl and the second from methyl alcohol.

In the next two preparations methyl and butyl salicylates were used in ethyl alcohol solution, taking care, however, to use an excess of ester over alkali. The results show that the butyl derivative was obtained pure, but with some difficulty, while the methyl could never be gotten up to the proper melting point. Slight ester transformation must have taken place in both cases, which was to be expected, since slight alcoholysis of the phenolates would take place. The details are given in Table III.

TABLE III.

	Solvent.		First crop.		Second crop.	
	Vo..	%.	Wt.	M. p. °C.	Wt.	M. p. °C.
Methyl salicylate in EtOH.....	35	70	1.06	122	liq.	...
	20	MeOH	0.85	125	0.13	111
	30	MeOH	0.71	126.5	0.11	118
	25	MeOH	0.61	126.5	0.07	123
	10	BuOH	0.34	126.5	0.08	90
Butyl salicylate in EtOH.....	25	75	1.22	86–9	emul.	...
	20	72	1.12	86–90	0.06	78
	25	MeOH	0.67	92	0.23	78
	13	EtOH	0.43	92	0.01	85

It appears that the transformation of the esters is minimized by using excess of the salicylic ester and that characteristic derivatives can be obtained, though not in very pure form.

In the case of the methyl salicylate derivative above, the attempt was made to change the methyl into the butyl derivative by adding a small amount of sodium to the butyl alcohol from which the last crystallization was made. The solution took on a purple color on addition of the compound to the sodium butylate solution. As shown by the melting point of the crystals that were obtained from this solution, no such reaction had taken place. The most probable explanation is that the de-

rivative is a strong enough acid to remove practically all of the sodium butylate from solution. An examination of the formula

$$NO_2C_6H_4CH_2OC_6H_4COOCH_3$$

shows the presence of the $—CH_2—$ group between two strongly negative groups. The nitro group may be concerned in the salt formation. Several other experiments of this kind were tried and all agreed with this in showing that these derivatives when once formed are not readily transformed by alcoholysis.

To show the effect of alcoholysis, or ester transformation, the following 3 experiments were tried. The salicylic acid esters were measured accurately into the alcohols in which 10% of sodium over that equivalent to the esters had been dissolved. These solutions stood for a time and were then heated, each with one g. of the reagent. The results are given below in the usual form.

TABLE IV.

	Solvent.		First crop.		Second crop.	
	Vol.	%	Wt.	M. p. °C.	Wt.	M. p. °C.
Methyl salicylate in BuOH.....	20	72	0.15	91	0.27	88
	8	60	0.12	91	0.01	86
Ethyl salicylate in MeOH.....	30	84	0.74	127.5	0.02	124
	15	EtOH	0.68	127.5	0.02	126
Butyl salicylate in MeOH.....	30	84	0.56	127	0.05	118
	13	EtOH	0.50	127.3	0.02	125

It appears, in every case, that the derivative obtained corresponds to the alcohol that is used as solvent and not to that originally present in the ester. The products obtained melted slightly low but close enough to the true melting point to be sure that the transformations had been practically complete.

Summary.

It has been found that *p*-nitrobenzyl esters may be used to identify salicylic esters provided no excess alkali is present. They are best made in the alcohol corresponding to the ester.

p-Nitrobenzyl ethers have been made of the following phenols and these properties studied:

o-Nitrophenol, m. p. 130°.
p-Nitrophenol, m. p. 187.4°.
2,4-Dinitrophenol, m. p. 248°.
o-Chlorophenol, m. p. 100°
p-Chlorophenol, m. p. 101.3°.
2,4,6-Tribromophenol, m. p. 163.5°.
Guaiacol, m. p. 63.6°.
m-Cresyl salicylate, m. p. 118°.
α-Naphthol, m. p. 140°.
β-Naphthol, m p. 106.5°.
Salol, m. p. 87°.
Methyl salicylate, m. p. 128.2°.
Ethyl salicylate, m. p. 125°.
Butyl salicylate, m. p. 92°.
o-Cresyl salicylate, m. p. 142.5°.

CLAREMONT, CAL., and BALTIMORE, MD.