The Poisonous Effect Exerted on Living Plants by Phenols.

By

Rodney H. True, Ph. D., and Carl G. Hunkel, B. S.[1])

(Conclud.)

If the introduction of this radicle into the phenol molecule increases its toxic effect to so considerable an extent, the question arises concerning its effect when introduced into other compounds.

By replacing one H of the benzene ring with an NO_2 group, we have nitrobenzene, a compound differing from the nitrophenols just studied by having none of its H hydrogens replaced by the OH group. In nitrobenzene, consequently, we can have no $\overset{+}{H}$ ions. Since the introduction of OH groups does not materially affect the toxicity of a compound dissociating but slightly, as resorcinol, we may compare the toxic effect of nitrobenzene with that of the nitrophenols which do not dissociate; i. e., when no H ions are present. This is realized when NaOH is added to the nitrophenols.

Nitrobenzene, $C_6H_5NO_2$.

Table XXXVI.

Begun Mar. 2, 8 : 25 P. M.

Gram-mol. per liter.	Length Mar. 3. 3 : 15 P. M.	Length Mar. 5. 3 : 30 P. M.	Condition.
1/1600	15,0 mm	14,5 mm	Dead.
	19,0 „	21,0 „	„ (?)
	16,5 „	17,0 „	„
	18,0 „	18,0 „	„
1/3200	15,5 „	15,5 „	„
	18,0 „	21,0 „	Alive.
	17,0 „	19,0 „	„
	17,0 „	20,0 „	„
1/6400	17,0 „	26,0 „	„
	15,0 „	20,0 „	„
	18,5 „	23,0 „	„
	18,0 „	23,0 „	„

A comparison of Tables XXXIII and XXXVI shows that the toxic value of para-nitrophenol + 1 NaOH is found to be somewhat greater than that of nitrobenzene, the latter, however, being still strongly poisonous. Its toxic action is about equal to that of ortho-nitrophenol + 1 NaOH (Table XXX).

Returning to the phenol compounds, we may ask, in view of the destructive results seen to follow the introduction of one NO_2 group into the phenol molecule, what may be the effect of the presence of a larger number of these groups. Unfortunately, dinitrophenol, $C_6H_3(OH)(NO_2)_2$, was not available, picric acid, trinitrophenol, only being at hand.

Trinitrophenol (Picric Acid) C_6H_2 (OH) (NO_2) (NO_2) $(NO_2) = 1 : 2 : 4 : 6$.

Table XXXVII.

Begun Dec. 8, 4 : 00 P. M.

Gram-mol. per liter.	Length Dec. 9. 11 : 40 A. M.	Condition, Dec. 10.
1/3200	16,5 mm	Dead.
	17,5 „	„
1/6400	26,0 „	Alive.
	22,0 „	Dead.
1/12800	36,0 „	Alive.
	30,0 „	„

Tables XXXVIII.

Sodium picrate.

Begun Jan. 24, 4 : 00 P. M.

Gram-mol. per liter.	Length Jan. 25. 11 : 00 A. M.	Length Jan. 26.	Condition.
1/400	15,0 mm	—	Dead. translucent.
	15,0 „	—	„
	16,5 „	—	„
	15,5 „	—	„
1/800	15,5 „	15,5 mm	Dead.
	16,0 „	26,0 „	Alive.
	15,5 „	15,5 „	Dead.
	17,0 „	29,0 „	Alive.
1/1600	15,5 „	—	Dead.
	14,5 „	—	„
	19,5 „	30,0 „	Alive.
	16,0 „	24,0 „	„

Picric acid, trinitrophenol, contains three NO_2 groups, and should the multiplication of these groups increase the toxic action in proportion, this compound should be decidedly more poisonous than the mono-nitrophenols. A comparison of Tables XXIX, XXXII and XXXVI shows that this is not the case.

Ostwald[1]) has shown that trinitrophenol behaves much like the strong acids. Solutions of one gram-molecule in 1024 liters are almost completely dissociated, and we should expect it to follow the rule of these substances. According to Table XXXVI, we see that it behaves toward lupines in a manner similar to that of a completely dissociated monobasic acid.[2]) Its toxic value agrees with that found for HCl and HNO_3, 1/6400. The toxic action appears, however, to be due in part only to H ions, the anion seeming also to possess poisonous properties. This appears on comparing the toxic value of the acid with that of its sodium compound. The toxic action of the anion is seen by comparing the concentrations of sodium picrate and sodium chloride first allowing growth. The toxic value of sodium picrate is 1/800,

---

[1]) Ostwald, Journ. für prakt. Chemie. XXXII. p. 354.
[2]) Kahlenberg and True, Bot. Gaz. XXII. p. 92.

that of sodium chloride about 1/4. Plainly, therefore, the anion
has very strong toxic properties towards lupines.

When the hydrogen of the OH group is replaced by CH_3,
we obtain from phenol, anisol, from pyrocatechol, the monomethyl
ether, guaiacol.

A n i s o l , $C_6H_5OCH_3$.

Table XXXIX.

Begun May 29, 4 : 45 P. M.

Gram-mol. per liter.	Length May 20. 2 : 35 P. M.	Length May 21.	Condition.
1/200	15,0 mm	14,5 mm	Dead.
	15,5 „	15,0 „	„
	15,0 „	15,0 „	„
	14,5 „	14,5 „	„
1/400	16,5 „	19,5 „	Alive.
	15,0 „	14,5 „	Dead.
	16,0 „	17,0 „	Alive. (?)
	18,5 „	25,0 „	„
1/800	15,0 „	14,5 „	Dead.
	15,0 „	18,0 „	Alive.
	15,5 „	17,5 „	„
	18,0 „	35,0 „	„

From this table, it appears that the toxic action of phenol
is approximately the same as that of its methylether, anisol,
about one gram-molecule in four hundred liters first permitting
growth. Filaments of S p i r o g y r a placed in solutions of this
substance were found after nearly twenty hours to survive first
in a concentration of about one gram-molecule in three hundred
liters, a some what weaker solution than the corresponding con-
centration of phenol producing the same results. In cells of
S p i r o g y r a killed in an anisol solution (1/200 gram-molecule
per liter) the chlorophyll loses its spiral form and collects in one
or more round masses, but the protoplast does not shrink away
markedly from the cell wall.

G u a i a c o l , $C_6H_4(OCH_3)(OH) = 1 : 2$.

Table XL.

Begun May 19, 4 : 30 P. M.

Gram-mol. per liter.	Length May 20. 2 : 30 P. M.	Length May 22. 3 : 30 P. M.	Condition.
1/400	15,0 mm	14,5 mm	Dead.
	15,0 „	14,5 „	„
	14,5 „	14,0 „	„
	16,0 „	18,0 „	„
1/800	22,0 „	42,0 „	Alive.
	20,0 „	28,0 „	„
	22,0 „	46,0 „	„
	17,0 „	18,0 „	„ (?)
1/1000	20,5 „	45,0 „	„
	15,0 „	15,0 „	Dead.
	16,0 „	20,0 „	Alive.
	17,0 „	20,0 „	„

394   True and Hunkel, The Poisonous Effect Exerted etc.

From the accompanying table, guaiacol is an active poison to lupines, the most concentrated solution allowing growth being that containing 1/800 gram-molecule per liter. being, therefore, more strongly toxic than anisol. It will be observed that here, as in the case of anisol, the ether has the same toxic value as the phenol from which it may be regarded as derived by replacing the H of an OH group with the CH_3 group.

Spirogyra filaments give results differing somewhat from those obtained with the lupines. As in the case of anisol, the alga is more tolerant toward the compound than are the lupines, the first solution in which majority of the filaments survived being 1/150 to 1/200 gram-molecule per liter. This is a stronger solution than the alga was able to withstand in the case of anisol, thus reversing the relative positions in the scale of toxicity as seen for the lupines.

Salicylic acid, $C_6 H_4$ (OH) (COOH) $= 1 : 2$, is both an acid and a phenol in its constitution, but the acid characteristics give it its most pronounced properties. The toxic value of salicylic acid has been determined by Kahlenberg and True.[1] The concentration first permitting lupines to survive was found in a solution containing 1/6400 gram-molecule per liter, the boundary observed, generally, for mono-basic acids undergoing complete dissociation. Ostwald[2] has shown that at the much stronger concentration of one gram-molecule in 1024 liters this acid dissociates 62,80 per cent. A materially greater degree of dissociation might be expected in the dilutions here used.

Ortho-oxy benzoic acid, salicylic acid, $C_6 H_4$ (OH) (COOH) $= 1 : 2$. Boundary-concentration, 1/6400 gram-molecule per liter.

Table XLI.

Sodium salicylate.

Begun May 21, 5 : 25 P. M.

Gram-mol. per liter.	Length May 22. 3 : 30 P. M.	Length May 21. 3 : 00 P. M.	Condition.
1/100	16,0 mm	20,5 mm	Alive.
	15,0 „	15,0 „	Dead.
	16,5 „	18,0 „	Alive.
	15,5	15,0 „	Dead.
1 200	15,5 „	15,0 „	„
	16,5 „	20,0 „	Alive.
	15,5 „	20,0 „	„
	16,0 „	35,5 „	„

[1] Kahlenberg and True. Bot. Gaz. XXII. p. 120. and MSS. results not yet published.

[2] Ostwald, W., Zeitschrift für physik. Chemie. Band II.

Table XLII.

Methyl salicylate.
Begun May 22, 4 : 25 P. M.

Gram-mol. per liter.	Length May 23. 2 : 30 P. M.	Length May 25. 4 : 00 P. M.	Condition.
1/800	15,0 mm	15,0 mm	Dead.
	15,0 „	15,0 „	„
	15,5 „	15,0 „	„
	15,5 „	15,0 „	„
1/1600	19,5 „	22,0 „	Alive.
	15,5 „	15,5 „	Dead.
	15,5 „	16,5 „	Alive.
	16,0 „	18,5 „	„

By replacing the acid hydrogen of salicylic acid with some harmless ion, we are able to obtain some idea of the intensity of the action of the anion, assuming relatively complete dissociation. The Na compound and methyl salicylate were studied in this connection and gave, as Tables XL and XLI show, quite different results. The low toxic value of the sodium salt would be expected, the hydrogen ions, found to constitute in general the markedly toxic agent in acids, being disposed of, practically harmless sodium appearing instead. The remaining toxic activity, a still appreciable quantity, is due to anions or to a possible residue of undissociated molecules or to both.

Methyl salicylate conducts itself somewhat differently. Its boundary-concentration is a much more strongly diluted solution than that of sodium salicylate. This greater toxicity is hardly to be attributed to the presence of the methyl group (CH_3). Probably hydrolytic dissociation takes place, forming methyl alcohol and salicylic acid. As is shown by the persistence of the characteristic odor, this hydrolytic splitting up is only partial. It would be expected that the salicylic acid thus split off would undergo electrolytic dissociation and a greater or less quantity of $\overset{+}{H}$ ions would appear in the solution, increasing proportionally its toxic action. It is probably, therefore, $\overset{+}{H}$ ions which render the solution of methyl salicylate so much more deadly than sodium salicylate.

In a recent work, Davenport[1] states that „by replacing one of the H atoms of phenol by COOH (or carboxyl), thus producing salicylic acid, the poisonous qualities are reduced". For the lupines and Spirogyra, this statement seems not to hold, as the following comparison of limit-concentrations shows. For lupines this limit is 1/6400 gram-molecule per liter of salicylic acid and 1/400 gram-molecule per liter of phenol; for Spirogyra the limit concentration for salicylic acid is about 1/2000

[1] Davenport, C. B., Experimental Morphology. Vol. I. 1897. p. 19.

gram-molecule per liter, and 1/200 gram-molecule per liter of phenol.

Sumary of Results.

In order to facilitate a comparison of the toxic values of the substances studied, we bring together in the following table their names and the boundary concentrations found for *Lupinus albus*:

Substance.	Formula.	Boundary Concentration.		
Mon-atomic Phenols.				
Benzophenol	$C_6 H_5 OH$	1/400	gram-mol. per liter.	
Benzophenol + 1 NaOH	·	1/400	,,	,,
Benzophenol + 1 NaCl		1/400	,,	,,
Benzophenol + 2 NaCl		1/400	,,	,,
Benzophenol + 3 NaCl		1/400—1/800		,,
Di-atomic Phenols.				
Pyrocatechol	$C_6 H_4 (OH) (OH)$ = 1 : 2	1/800	,,	,,
Resorcinol	$C_6 H_4 (OH) (OH)$ = 1 : 3	1/200	,,	,,
Resorcinol + 1 NaOH		1/400	,,	,,
Resorcinol + 2 NaOH		1/800	,,	,,
Hydroquinone.	$C_6 H_4 (OH) (OH)$ = 1 : 4	1/1600	,,	,,
Tri-atomic Phenols.				
Pyrogallol (fresh sol.)	$C_6 H_3 (OH) (OH) (OH)$ = 1 : 2 : 3	1/1600	,,	,,
Pyrogallol (old sol.)		1/6400	,,	,,
Phloroglucin	$C_6 H_3 (OH) (OH) (OH)$ = 1 : 3 : 5	1/400	,,	,,
Derivatives.				
Ortho-cresol	$C_6 H_4 (CH_3) (OH)$ = 1 : 2	1/800	,,	,,
Ortho-cresol + 1 NaOH		1/400	,,	,,
Meta-cresol	$C_6 H_4 (CH_3) (OH)$ = 1 : 3	1/800	,,	,,
Meta-cresol + 1 NaOH		1/400	,,	,,
Para-cresol	$C_6 H_4 (CH_3) (OH)$ = 1 : 4	1/1600	,,	,,

Substance.	Formula	Boundary Concentration.
Para-cresol + 1 NaOH		$1/1600$ gram-mol. per liter
Carvacrol	$C_6H_3(CH_3)(OH)(C_3H_7)$ $= 1 : 2 : 4$	$1/3200$,, ,,
Carvacrol + 1 NaOH		$1/3200$,, ,,
Thymol	$C_6H_3(CH_3)(OH)(C_3H_7)$ $= 1 : 3 : 4$	$1/3200$,, ,,
Thymol + 1 NaOH		$1/3200$,, ,,
Ortho-nitrophenol	$C_6H_4(OH)(NO_2)$ $= 1 : 2$	$1/12500$,, ,,
Ortho-nitrophenol + 1 NaOH		$1/3200$,, ,,
Para-nitrophenol	$C_6H_4(OH)(NO_2)$ $= 1 : 4$	$1/6400$,, ,,
Para-nitrophenol + 1 NaOH		$1/6400$,, ,,
Tri-nitrophenol	$C_6H_2(OH)(NO_2)(NO_2)$ $(NO_2) = 1 : 2 : 4 : 6$	$1/3200$,, ,,
Tri-nitrophenol + 1 NaOH		$1/800$,, ,,
Nitrobenzene	$C_6H_5NO_2$	$1/3200$,, ,,
Anisol	$C_6H_5(OCH_3)$	$1/400$,, ,,
Guaiacol	$C_6H_4(OCH_3)(OH)$ $= 1 : 2$	$1/800$,, ,,
Orcinol	$C_6H_3(CH_3)(OH)(OH)$ $= 1 : 3 : 5$	$1/400$,, ,,
Salicylic acid	$C_6H_4(OH)(COOH)$ $= 1 : 2$	$1/6400$,, ,,
Sodium salicylate		$1/100—1/200$,,
Methyl salicylate		$1/1600$,, ,,

In reviewing the foregoing, we find, as far as our knowledge of the dissociation of the phenylic compounds permits us to draw conclusions, that, except in isolated instances, electrolytic dissociation plays but a very subordinate rôle in determining the toxic properties of the substances. Picric and salicylic acids strongly dissociate and become powerfully poisonous by virtue of the H ions, in great measure. Pyrogallol and probably methyl salicylate first undergo other molecular changes, after which their products dissociate electrolytically. Here the H ions may account for much of the toxic action. In the cresols and mono-nitrophenols, electrolytic dissociation seems to exert a pronounced influence. Some phenols are comparatively weak in their integrity, but quickly change to substances containing constituents even more fatal than

H ions. Pyrocatechol and especially hydroquinone are of this class.

Certain radicles seem to have specific properties when intro-
duced into the molecule, modifying the toxic value. The number
of hydroxyl groups (OH) present seems to have little influence
on the toxic action of the phenols, as in the series: benzophenol
(1 OH), resorcinol (2 OH), and phloroglucin (3 OH). The intro-
duction of the methyl group (CH₃) into the benzene nucleus
increases the toxicity to a considerable, but rather variable,
degree, as in the cresols, less plainly in orcinol. The introduction
of the isopropyl group (—CH[CH₃]₂) into the cresols increases
the toxic value of these substances, as carvacrol and thymol. The
presence of one or more nitro groups (NO₂) increases the toxic
action to a great degree; mono- and tri-nitrophenols. An increase
in the number of the NO₂ groups present does not seem to
increase the toxic action. When the H of an OH group is repla-
ced by a (CH₃) group, little influence seems to be exerted on
the toxic action, e. g., anisol and guaiacol. The carboxyl group
(COOH) brings with it a degree of toxicity corresponding directly
to the degree of dissociation and the number of H ions it
affords; salicylic acid.

Nachträgliche Bemerkung

zu meinem Aufsatz in Nr. 9 dieses Bandes. p. 298: Das
Leuchten des *Ceratium tripos* hat soeben J. Reinke (Wissenschaft-
liche Meeresuntersuchungen. Band III. 1898. p. 39—41) näher
studirt. Dasselbe wird nicht durch Bakterien verursacht.

Prof. Dr. Ludwig - Greiz.

Instrumente, Präparations- und Conservations-Methoden etc.

Der Sudan III und seine Verwendung in der botanischen Mikrotechnik.

Von
Dr. **Luigi Buscalioni**
in Rom.

Der Farbstoff Sudan III wurde seit einiger Zeit in der
medicinischen und zoologischen Mikrotechnik sehr empfohlen, um
die Fette zu färben. Jedoch ist meines Wissens dieses Reagenz
in der botanischen Mikrotechnik bis jetzt nicht angewendet worden.
Ich habe daher damit Versuche angestellt. Aus meinen eingehenden