CONCLUSION

A useful, simplified kinetic description of nalidixic acid's fate in man has been presented for three of four dosage forms. These results clearly indicate that the pharmacokinetic analysis of a drug must include provision for the biopharmaceutic factors of the dosage form of the drug. When correction is allowed for these modifying factors, the parameters of elimination kinetics become meaningful, as for example, the constancy of k_a.

Lag times and availability rates varied significantly between the dosage forms as expected. However, the differences in behavior between the micropulverized powder in a capsule and in a Caplet are not extreme; they show the same k_A but different lag times. This effect is not due to a capsule as such (the short lag time of the sodium salt shows that a capsule can release its contents quite rapidly), but it is most likely due to the fact that the Caplet contains adjuvants that bring about rapid dispersion of the drug.

The model failed completely to describe the pharmacokinetics of nalidixic acid when administered orally as a coarse powder. A model to describe this slowly absorbed dosage form is the subject of continuing research.

REFERENCES

(1) Nelson, E., This Journal, **50**, 181(1961).
(2) Wagner, J., *ibid.*, **50**, 359(1961).
(3) Levy, G., in "Prescription Pharmacy," Sprowls, J. B., Jr., ed., J. B. Lippincott Co., Philadelphia, Pa., 1963, Chapter 2.
(4) McChesney, E. W., *et al.*, *Toxicol. Appl. Pharmacol.*, **6**, 292(1964).
(5) Goss, W. A., and Deitz, W. H., *Bacteriol. Proc.*, **1963**, 93.
(6) Levy, G., *op. cit.*, p. 69.

Structure–Chromogenic Activity Relationship of Phenolic Compounds with Ehrlich Reagent

By GOVIND J. KAPADIA, JAMES R. MOSBY, GEETA G. KAPADIA*, and THEODORE B. ZALUCKY

Over 100 phenolic compounds have been hitherto tested for their chromogenic ability with a modified Ehrlich reagent. A simple paper spotting technique has been utilized for this study. Mono-, di-, and trihydroxyphenols have been tested, and the possible relationship of color and structure has been investigated. Color tests were observed in the case of many compounds, with resorcinol, phloroglucinol, and several of their derivatives yielding relatively sensitive chromogenic reactions. The chromogenic effect of the alkyl substituent, etherification, and esterification of phenolic hydroxyl group(s) of resorcinols has been studied. The color reactions of three types of carbonyl-substituted resorcinols previously reported were investigated further by examining the effect of electron-donating groups in mitigating the adverse effect of the carbonyl group. Several naturally occurring phenolic compounds were also tested by the same technique. The modified Ehrlich reagent is a useful phenolic chromogen and may be applicable as a differentiating reagent for some of these compounds.

Ehrlich reagent (1), consisting of an acidic solution of *p*-dimethylaminobenzaldehyde (PDAB), is generally used in chromogenic analysis for the detection (2–5) of indole derivatives in plant and animal extracts. Spraying this reagent on paper chromatograms containing indole compounds produces purple, blue, and red spots. According to Feigl (2), even pyrrole and its derivatives that have intact CH-group in the α- and β-position relative to the cyclic NH-group will react with PDAB to yield colored products. It was indicated further that aliphatic amines condense with this aldehyde and produce various colored condensates—usually yellow, orange-red, and brown.

Morton (6) found that the Ehrlich reagent was applicable for the chromogenic analysis of phloroglucinol, pyrogallol, orcinol, and resorcinol. The colors were similar to those produced by indoles and pyrroles under conditions of the Ehrlich test. Since then the effect of this reagent on a number of phenolic compounds has been reported (8–12). The studies of these workers revealed that generally most of the phenols did not appear to react on the paper chromatograms with the reagent. Excluding some cinnamic acid derivatives, all that did so were resorcinol or phloroglucinol derivatives. Phloroglucinol-derived compounds reacted instantaneously, whereas the resorcinol-derived compounds developed colors after a while.

Received August 19, 1963, from the Department of Pharmacognosy and Natural Products, College of Pharmacy, Howard University, Washington, D. C.
Accepted for publication September 3, 1964.
This investigation was supported in part by grant MH 06905-01 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
Presented to the American Pharmacognosy Society, Chapel Hill, N. C., meeting, July 1963. (See *Reference 7* for published abstract.)
* Present address: Department of Cardiology, Georgetown University Medical Division, District of Columbia General Hospital, Washington, D. C.

Among the three resorcinol carboxylic acids, Acheson and co-workers (9) observed that γ-resorcylic acid gave a pale blue color in an unspecified amount. However, Steelink (11), McGeer and co-workers (12), and later Acheson and Turner (13) reported that all the three resorcylic acids yielded no color reaction. Although no other carbonyl-substituted resorcinols were examined, Acheson and Turner (13) concluded that this substituent in general deactivated the ring and thus inhibited the color reaction. Phloroglucinolcarboxylic acid and 2,4,6-trihydroxyacetophenone, however, gave pink products (11), an indication that additional activation by a phenolic group could mitigate the adverse effect of the carboxyl or other carbonyl group.

Kapadia and Zalucky (14) examined resorcinol derivatives with various electron-withdrawing carbonyl substituents. Tests on such phenols, with hydroxyl groups in 2,4-, 2,6-, and 3,5-positions, revealed varying chromogenic activity. 2,6-Dihydroxycarbonyl-containing phenols showed the most sensitive color reaction; pink to violet coloration was noticeable even in an amount of 1 mcg. The compounds in which hydroxyl groups were in 3- and 5-positions showed the least sensitive color test, and the colored products were yellowish.

Recently, when the present work was in progress, Acheson and Turner (13) reported the reaction involved in the formation of purple compounds from Ehrlich reagent and resorcinol and 2-methylresorcinol. In both the cases amphoteric purple compounds were isolated. Analysis of the colored products showed that equimolar proportions of reactants combined with loss of some hydrogen. On the basis of these, they proposed the structure of the colored products. They also found that a resorcinol required a free 4- or 6-position if it was to give a color with the reagent. These and some previous workers (8, 9, 11, 13), however, provided no data on the amount of test compound employed in their studies. Others (12), who indicated the quantity, made the color observations after chromatographing a single amount (20 mcg.) of the test substance on paper.

This work was undertaken to clarify and extend the findings of the previous investigators and to observe the color reactions by performing spot tests on varying amounts of phenolic compounds with Ehrlich reagent, modified as reported here. In resorcinol and phloroglucinol derivatives, additional studies were carried out to determine the influence of various substituents on the chromogenic tests. Finally, some of the naturally occurring phenolics related to resorcinol or phloroglucinol were tested to determine the usefulness of the reagent for their detection and tentative identification.

In studying the color reactions of the phenols, spot tests were preferred because of their advantages over the paper chromatographic methods used by previous workers (9, 11, 12). In the latter methods, different compounds might tail and spread on a chromatogram to a different extent. Intensities of the colored spots, which depend on the sensitivity of test compounds, might vary because of this fact. Results from these could be less suitable, particularly for comparative studies that have to be made to elucidate structure-chromogenic activity relationship. Spot tests on paper not only overcome this disadvantage, but also they are rapid, economical, and provide more useful data for chromogenic studies that might be followed, if necessary, by chromatographic methods.

In carrying out the tests, generally six different amounts of phenolic compounds varying from 1 to 100 mcg. were spotted on paper. A definite quantity of the reagent then was applied to each test compound. Spraying of the reagent was also tried; but for the determination of the sensitivity of the test, spotting of the reagent over the phenolic compound was preferred. In this manner it was possible to apply a definite quantity of the reagent and minimize spreading of the compound.

EXPERIMENTAL

Test Compounds.[1]—All compounds used in this study were obtained from commercial sources or were provided by other investigators. Compounds containing impurities as evidenced by determination of melting or boiling point were discarded. A list of the compounds is given in Table I together with the source for each.

Ehrlich Reagent.—Ehrlich reagents of varying composition have been used by various workers (1–3, 9, 11, 15). In addition to varying the proportions of PDAB and hydrochloric acid, some investigators have used aqueous solutions; other have used alcoholic or acetone solutions. The composition of reagent used in the study was similar to that reported by Reddi and Kodicek (16), except for the acid concentration. It was prepared by dissolving 0.5 Gm. of PDAB in 90 ml. of absolute alcohol; 2.8 ml. of concentrated hydrochloric acid was added, and the volume was made to 100 ml. with absolute alcohol.

[1] The authors express sincere appreciation to Drs. H. Sieper and F. Korte, University of Bonn, Bonn, Germany, for samples of cannabidiol, cannabinol, and tetrahydrocannabinol; T. Seshadri, University of Delhi, Delhi, India, for orsellinic acid and orcylaldehyde; R. Mechoulam, Weizman Institute of Science, Israel, for cannabidiol bisdinitrobenzoate; A. Penttilä, Medica, Ltd., Helsinki, Finland, for 2,4,6-trihydroxybutyrophenone, 2,4,6-trihydroxy-3-methylbutyrophenone, and o-desaspidinol; O. Schultz, University of Kiel, Kiel, Germany, for cannabidiol acid diacetate; and G. Johnson, Colorado State University, Boulder, for hydroxyhydroquinone and 2,4,5-trihydroxybenzoic acid.

TABLE I.—RESULTS OF SPOT TESTS ON PHENOLIC COMPOUNDS

Compd.	Source[a]	Color[b]	Amt. of Test Compd., mcg.[c]
Monohydroxy Phenolic Compd.			
Phenol	B	Pale pink	100
o-Cresol	F	Light orange	100
m-Cresol	F	Light orange	100
p-Cresol	F	Light orange	100
2,3-Dimethylphenol	KK	Light pink	50
3,4-Dimethylphenol	KK	Light violet	50
Thymol	F	Pale pink	10
α-Naphthol	F	Light yellow	5
β-Naphthol	F	Brownish-violet	5
o-Hydroxycinnamic acid	A	Light green	100
m-Hydroxycinnamic acid	A	Light yellow	100
p-Hydroxycinnamic acid	A	Light purple	100
4-Hydroxy-3-methoxycinnamic acid	A	Pale green	50
3-Cyanophenol	A	Pale yellow	100
Salicylaldehyde	F	Pale brownish-pink	100
Salicylic acid	F	No color	...
Eugenol	F	No color	...
Vanillin	F	No color	...
Guaiacol	F	No color	...
Syringaldehyde	A	Pale yellow	10
Syringic acid	A	Brownish-pink	10
4-Hydroxystilbene	A	Pale yellow	10
Dihydroxy Phenolic Compd.			
Catechol	F	Light pink	5
3-Methylcatechol	A	Light pink	5
4-Methylcatechol	A	Light pink	5
3-Methoxycatechol	A	Light pink	5
3,4-Dihydroxybenzaldehyde	A	Light yellow	10
3,4-Dihydroxybenzoic acid	A	Light yellow	10
3,4-Dihydroxy-5-methylbenzaldehyde	KK	Light yellow	10
3,4-Dihydroxy-5-methoxybenzaldehyde	KK	Light yellow-green	10
4-Chlorocatechol	A	Light brown	10
Epinephrine	F	Light pink	10
Hydroquinone	F	Light pink	5
2,5-Dihydroxyacetophenone	E	Light yellow	50
2,5-Dihydroxybenzaldehyde	A	Light brown	50
2,5-Dihydroxy-*p*-benzoquinone	KK	Pink	1
Resorcinol	B	Violet	1
2-Methylresorcinol	A	Violet	1
4-Ethylresorcinol	A	Violet	2
4-*n*-Propylresorcinol	A	Violet	2
4-*n*-Hexylresorcinol	C	Light violet	10
4-Cyclohexylresorcinol	A	Violet	5
4-Benzylresorcinol	A	Violet	2
4-*n*-Dodecylresorcinol	A	Light violet	10
4-*n*-Hexadecylresorcinol	A	Light violet	50
4-*n*-Octadecylresorcinol	A	Light brown-violet	100
5-Methylresorcinol (Orcinol)	F	Pink	1
5,5-Dimethyldihydroresorcinol	E	Light orange	1
Resorcinol monomethyl ether	E	Light pink	5
Resorcinol monoethyl ether	E	Light pink	5
3-Methoxy-2-methylphenol	A	Pale yellow-green	5
Resorcinol dimethyl ether	E	No color	...
Resorcinol diethyl ether	E	No color	...
Resorcinol monoacetate	F	Light purple	5
Resorcinol monobenzoate	A	No color	...
Resorcinol diacetate	E	Pale green	100
4-Chlororesorcinol	F	Violet	2
4-Bromoresorcinol	A	Violet	2
2,4-Dihydroxyphenylsulfide	A	Green-blue	1
1,3-Dihydroxynaphthalene	A	Brilliant blue	1
1,5-Dihydroxynaphthalene	A	Green-brown	2
2,3-Dihydroxynaphthalene	A	Light green	2
2,7-Dihydroxynaphthalene	E	Light violet	1
Trihydroxy Phenolic Compd.			
Pyrogallol	F	Light violet	2
2,3,4-Trihydroxyacetophenone	A	Pale green	50
3,4,5-Trihydroxybenzoic acid (gallic acid)	F	Pale yellow	50
Hydroxyhydroquinone	J	Light green-blue[d]	1
2,4,5-Trihydroxybenzoic acid	J	Yellow	50
2,4,5-Trihydroxybutyrophenone	A	Yellow	50
Phloroglucinol dihydrate	F	Brilliant pink	1
2,4,6-Trihydroxytoluene	A	Pink	1
2,4,6-Trihydroxyacetophenone	A	Pink	1
2,4,6-Trihydroxybenzaldehyde	A	Pink	1
2,4,6-Trihydroxybenzoic acid	A	Pink	1
2,4,6-Trihydroxybutyrophenone	P	Pink-violet	1
2,4,6-Trihydroxy-3-methylbutyrophenone	P	Pink-yellow[e]	2

continued overleaf

TABLE I.—CONTINUED

Compd.	Source[a]	Color[b]	Amt. of Test Compd., mcg.[c]
Some Naturally Occurring Phenols and Derived Compd.			
Sesamol	A	Blue	1
Cannabinol	K	Pale pink-brown	5
Tetrahydrocannabinol (synthetic)[f]	K	Pale bluish-brown	5
Cannabidiol	K	Bluish-green	1
Cannabidiol acid diacetate	S	Light green-yellow	100
Cannabidiol bis-3,5-dinitrobenzoate	ME	Yellow	50
o-Desaspidinol	P	Light yellow-pink[g]	1
Ellagic acid	A	Pale pink	10
Usnic acid	A	Pale pink	100
Phloretin	M	Pink-violet	1
Phloridzin	A	Light pink-yellow	5
o-Orsellinic acid	SE	Light pink	1
o-Orcylaldehyde	SE	Light pink	5
Brazilin	KK	Light pink	2

[a] A, Aldrich Chemical Co.; B, J. T. Baker Chemical Co.; C, City Chemical Corp.; E, Eastman Organic Chemicals; F, Fisher Scientific Co.; J, Dr. G. Johnson; K, Drs. F. Korte and H. Sieper; KK, K & K Laboratories; M, Mann Research Laboratories; ME, Dr. R. Mechoulam; P, Dr. A. Penttilä; S, Dr. O. E. Schultz; SE, Dr. T. R. Seshadri. [b] Color shade was recorded for the lowest of the six different amounts of the test compound used. Shades of colors like blue, violet, purple, orange, yellow, pink, and green correspond to the names used in "Reinhold Color Atlas" (17). According to this publication, the order of a shade like blue is given as follows: vivid blue, blue, light blue, and pale blue. Mixed colors like pink-violet, brownish-violet, etc., are personal observations. [c] Spot tests on paper on six different quantities 1, 2, 5, 10, 50, and 100 mcg. of each compound were performed, and the lowest amount of the compound that gave a noticeable color reaction was recorded. [d] Gradually becomes dark brown-violet. [e] Gradually becomes pink-violet. [f] Synthetic THC, m.p. 62–63° was used. In this compound the alicyclic double bond is conjugated with olivetol ring. [g] Gradually becomes pink.

TABLE II.—COLOR REACTIONS OF SOME PARENT PHENOLS WITH EHRLICH REAGENT

Compd.	Color[a]	Sensitivity,[b] mcg.	Spectral Data			
			$\lambda_{max.1}$	ϵ_1	$\lambda_{max.2}$	ϵ_2
Resorcinol	Violet	1	566	3811	392	4052
Naphthoresorcinol	Blue	0.1	610	19058	...	...
Pyrogallol	Light violet	2	564	3647	388	3713
Hydroxyhydroquinone	Green-blue	1	594	10423	...	...
Phloroglucinol dihydrate	Pink	0.05	538	24237	...	...

[a] Color of the lowest amount detected by spot test on paper. [b] Minimum amount of the compound that showed a colored spot.

Test Procedure.—Solutions of test compounds were prepared in absolute ethyl alcohol. The solutions were spotted with a micropipet on a Whatman No. 1 paper strip. The diameter of each spot was confined to 3–4 mm.

Generally, six separate spots, each containing 100, 50, 10, 5, 2, and 1 mcg. of test compound were made. In some cases, where a sensitive color reaction was observed in a preliminary test, spotting of the higher amounts of 100 and 50 mcg. was omitted. Two microliters of the reagent (equivalent to 10 mcg. of PDAB) then was applied on the dried test spot, and the paper was placed in an oven at 100° for 1 minute. With some compounds, the color development was noticeable almost immediately at room temperature, but the intensity of the color was generally increased by transferring to the oven. The results noted were those of the color observed after heating at 100° for 1 minute. Where a distinct color increase was noticeable during a period of 12 hours, the spot test was repeated. To verify the increase in color, the aged and the new test spots were compared and increases, if any, recorded (Table I).

In some compounds that were colored or yielded light coloration on heating, a blank spot of each concentration of the test substance was made. Reagent was not applied to this spot. Two microliters of the reagent also was spotted separately. After comparing the colors of the separate test compound and reagent controls to the color of these combined, the differences were recorded. The recorded colors were those of the lowest concentration of the compound yielding a noticeable coloration. The reagent blank was pale yellow.

To determine the minimum amounts in which naphthoresorcinol (1,3-dihydroxynaphthalene) and the three parent compounds—*viz.*, resorcinol, phloroglucinol, and hydroxyhydroquinone—could be detected, they were tested with 2 μl. of the reagent in concentrations lower than 1 mcg. Results are recorded in Table II. It was possible to detect some other compounds, particularly phloroglucinol derivatives that yielded sensitive reactions in amounts less than 1 mcg. In the present studies, however, the testing of these compounds up to this quantity has been limited.

Absorption Spectra of Colored Products.—The spectra of the colored products from the five parent phenolic compounds were determined by the reaction of the phenols and PDAB in acidic alcohol. Five milliliters of an alcoholic solution containing 0.1 mmole of the test compound and 1% hydrogen chloride was mixed with 5 ml. of an alcoholic solution containing 0.2 mmole of PDAB. The mixture was heated on a water bath at 65° for 3 minutes. It was cooled to room temperature, and volume was made to 25 ml. by addition of ethanol containing 1% HCl. An alcoholic solution containing 0.2 mmole of PDAB

and 1% HCl was used as a blank. The absorption spectra of the colored solutions were determined in the visible region between 800 to 360 mμ wavelength using a Beckman DB spectrophotometer and a Sargent SRL recorder. The $\lambda_{max.}$ and extinction coefficient (ϵ) values are recorded in Table II.

Color Reactions of 4-Alkylresorcinols.—For comparing color reactions of 4-alkyl substituted resorcinols, equivalent molar concentrations of each compound were utilized. After heating for 1 minute at 100° with 2 μl. of the reagent, the color reaction of resorcinol (11 mcg.) was compared simultaneously to its 4-alkyl substituted derivative. Similar color comparisons were also made to some of the appropriate 4-alkyl derivatives. Amounts of the compounds employed and the colors observed are recorded in Table III. To confirm visual observations, the intensities of the colored spots were recorded using a Photovolt model 525 densitometer consisting of the Photometer model 501-A and transmission density unit 52-C. A round 2-mm. aperture and a 570-mμ filter were used. Average transmission readings are recorded in Table III.

TABLE III.—AVERAGE PERCENTAGE TRANSMISSION OF COLORED SPOTS OF 4-ALKYLRESORCINOLS

Compd.	Mol. Wt.	Amt. Spotted, mcg.	Transmission, % at 570 mμ
Resorcinol	110	11	36
4-Ethylresorcinol	138	14	39
4-*n*-Propylresorcinol	152	15	41
4-Cyclohexylresorcinol	192	19	59
4-*n*-Hexylresorcinol	194	19	70
4-Benzylresorcinol	210	21	53
4-*n*-Dodecylresorcinol	278	28	72
4-*n*-Hexadecylresorcinol	337	34	96
4-*n*-Octadecylresorcinol	362	36	98

RESULTS AND DISCUSSION

The results of the spot tests (Table I)[2] indicate that several phenols give color tests on paper with the modified Ehrlich reagent. Among these, many resorcinol and phloroglucinol derivatives yield distinct pink to violet colors in fairly low quantities. Phloroglucinol itself gives a coloration more intense than resorcinol. As indicated in Table II, it can be detected (as dihydrate) in quantities of 0.05 mcg., whereas the latter compound does not show a distinct color test in amounts lower than 1 mcg. In contrast to the color reaction of resorcinol, naphthoresorcinol (1,3-dihydroxynaphthalene) gives a characteristic blue coloration and can be detected in an amount as low as 0.1 mcg. Fusion of a benzene ring to a resorcinol molecule potentiates the color reaction. As a result of the extension of conjugation in the molecule, a bathochromic shift is observed in the maximum absorption of the spectrum from 566-mμ wavelength of violet-colored product of resorcinol to 594-mμ wavelength of a blue product of naphthoresorcinol (Table II).

The color reactions of the three trihydroxyphenols —*viz.*, pyrogallol, hydroxyhydroquinone, and phloroglucinol—with the modified Ehrlich reagent reveal the varying influence of additional hydroxyl group in a resorcinol molecule. An introduction of this group in the 2-position results in some reduction in sensitivity and intensity of the color test, as shown by the reaction of pyrogallol. Results of hydroxyhydroquinone indicate that the addition of a hydroxyl group in the 4-position of a resorcinol molecule yields a blue product. Compared to the violet product of resorcinol, the absorption maximum of this blue compound indicates a bathochromic shift. The color reaction of phloroglucinol reveals the influence of the addition of a hydroxyl group in the 5-position. Although this is accompanied by a considerable increase in sensitivity and intensity of the reaction, the maximum absorption of the colored product is at a lower wavelength than that of the product of resorcinol and PDAB.

Phenolic compounds such as monohydroxyphenols and catechol derivatives give the spot test, as shown in Table I. However, the intensity of color is low; generally, a higher concentration is required for a distinct color formation. In connection with reactions on mono- and dihydroxyphenols, the potentiating influence of a fused benzene ring is evidenced further by noting the spot test results with β-naphthol as well as those with 1,5- and 2,3-dihydroxynaphthalenes. These compounds also show relatively sensitive color tests, although the resulting colored products are not distinctly pink or violet. On the other hand, a light violet color formation is noticeable with 2,7-dihydroxynaphthalene in a 1-mcg. quantity. This suggests that the presence of a second hydroxyl group in the 7-position of β-naphthol results in further increase in sensitivity of the color test of the latter compound.

It is interesting to note the chromogenic reaction of 2,5-dihydroxy-*p*-benzoquinone. The compound gives a distinct pink color reaction in at least 1-mcg. quantity. The molecule might be considered to comprise two resorcinol moieties, each of which has one of the hydroxyl groups in a keto form. Comparison of the color test on this compound and resorcinol indicates that, although the latter shows violet coloration, the pink of the former compound appears to be more intense.

Color Reactions of Some Alkyl Resorcinols.—Monoalkyl resorcinols that have methyl or ethyl substitutents in the 2,4,- or 5-positions have been studied. The positive color reactions of these compounds in fairly low amounts are expected from the findings of Acheson and Turner (13). However, the results recorded in Table III indicate that, with an increase in length of the alkyl chain in the 4-position, there appears to be a diminution of chromogenic intensity. It is also indicated that this is not necessarily due to the increase in molecular weight. The diminution of the color intensity might be due to the decrease in polarity. The increase of the alkyl chain brings about decrease in solubility, thereby creating less molecular reaction and resulting in slow reaction rates and a decrease in formation of colored products.

Among the dialkyl-substituted derivatives, cannabidiol (CBD) (Fig. 1), the phenolic constituent of *Cannabis sativa*, yields a sensitive reaction. This compound is a 2,5-dialkyl derivative of resorcinol. Its positive reaction indicates that blocking of positions 2 and 5 of a resorcinol molecule by alkylation does not inhibit the color reaction as in the case of the 4,6-disubstituted derivative tested by Acheson and Turner (13). Furthermore, the presence of

[2] Over 100 phenols and derived compounds have been tested (7). Of these, 90 have been reported here.

cyclohexenyl and *n*-pentyl substituents on positions 2 and 5, respectively, appears to result in no inhibition of the chromogenic test as found in higher 4-alkyl homologs. The bluish-green shade of the colored product, compared to the normal pink-violet coloration resulting from the reaction of resorcinols and PDAB, suggests a bathochromic shift in the spectrum of the compound. This might be rationalized as being due to rearrangements occurring in the cyclohexenyl and the allylic side chain to yield in the colored product a system that would be in conjugation with the benzene ring of the molecule.

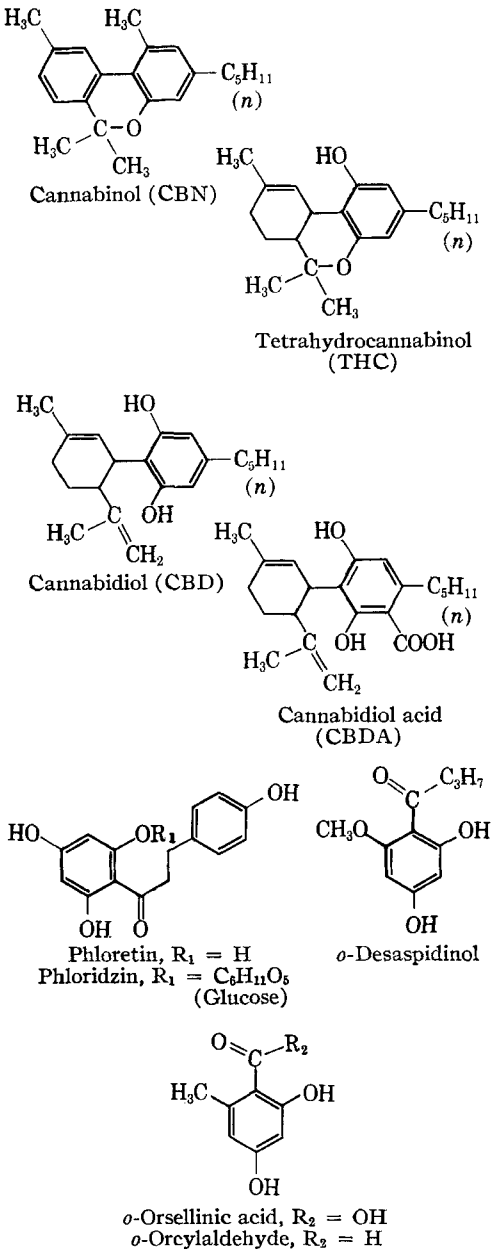


Fig. 1.—Structures of some naturally occurring and derived phenolic compounds. [Some doubt exists regarding the position of the double bond in the cyclohexene ring of THC, CBD, and CBDA, although it is not conjugated with the benzene ring (21). Recently, Mechoulam and Shvo (22), Gaoni and Mechoulam (23), and Mechoulam (24) have assigned structures for CBD, THC, and CBDA, respectively, as in Fig. 1. In synthetic THC, m.p. 62–63°, which was tested, the alicyclic ring is conjugated with olivetol ring.]

Effects of Etherification of Resorcinols.—Blocking of both the hydroxyl groups of resorcinol by etherification would result in loss of phenolic character and is expected to be accompanied by diminution of the color reaction. This is evidenced by the negative tests of resorcinol dimethyl and diethyl ethers. However, when only one of the phenolic hydroxyl groups is etherified—as in resorcinol monomethyl and monoethyl ethers—and although a positive color test is observed, there is reduction in sensitivity, and light pink products are formed. The reaction of sesamol (hydroxyhydroquinone methylene ether), on the other hand, suggests that blocking of one of the hydroxyl groups by a methylene ether linkage with an adjacent phenolic group does not result in reduction of sensitivity.

The inhibitory effect of etherification of one of the phenolic hydroxyl groups of resorcinol is also noticeable in the reduced sensitivity and light intensity of coloration of cannabinol (CBN) and synthetic tetrahydrocannabinol (THC), m.p. 62–63° (18) (Fig. 1). On the other hand, in the molecule of CBD both the phenolic hydroxyl groups are free, and it yields a sensitive reaction.

Concerning the chromogenic activity of CBD the indication might be made that the sample of this compound left to stand at room temperature may not yield the color reaction shown in Table I and reported in our preliminary communication (19). The first tests were performed in March 1963, immediately after the authentic sample (kindly provided by Drs. Sieper and Korte) was received. The sample was then left at room temperature (75–95° F.)[3] and was tested again in July 1963. In the subsequent tests, however, no characteristic blue-green color was observed as originally. In the later tests, only a pale yellow-green was noticeable in a 10-mcg. quantity. This suggests that certain changes in the molecule affect the chromogenic activity of the compound. It also might indicate that Beam's reagent, which is considered specific for CBD (20, 21), yields a purple color reaction with the stored sample. Therefore, it is not possible to detect the change, if any, that occurs in the molecule of stored CBD by using Beam's reagent.

The variation of the color reaction of stored and nonstored samples of CBD with our reagent may be useful in the detection of change that may occur in the molecule of CBD. The color test thus appears to provide a rapid, inexpensive, and simple method to detect such a change.

The usefulness of the PDAB reagent for the detection of CBD in *Cannabis* extract was confirmed by paper chromatography of a purified extract of Lebanese hashish. The procedure of Davis *et al.* (21) was followed for the development of the chromatogram. Modified Ehrlich reagent was used for spraying, and the color was developed as usual. A

[3] The stored compound melted at 64–66° and showed no distinct difference in the melting point from provided authentic sample (65–66°). It could not be examined further due to a limited amount at our disposal.

bluish-purple spot was observed[4] at the CBD R_f location. This spot was also identified as CBD by the other chromogenic reagents (21). The usefulness of the reagent for identification of CBD in the presence of THC was revealed.

Color Reactions of Some Resorcinol Esters.—Testing of resorcinol esters reveals that a color reaction is observed in resorcinol monoacetate, although the sensitivity is decreased. However, under similar conditions, the monobenzoate of resorcinol yielded no colored product with the Ehrlich reagent used in this work. If a positive test of the monoacetate ester of resorcinol can be explained as being due to the hydrolysis of the acetyl linkage under acidic medium, obviously resorcinol monobenzoate is relatively stable under the test conditions.

Concerning the relative stability of acetate and benzoate esters of resorcinols, Bergmann and Dangschat (25) reported that 2-acetoxy-4-benzoyloxybenzoic acid suffered only partial hydrolysis under acidic conditions to 4-benzoyloxysalicylic acid. This suggests that the acetate ester was less stable than the benzoate. Bradley and co-workers (26) synthesized 5-*O*-benzoyldelphinidin chloride at room temperature, starting with 2-*O*-benzoylphloroglucinaldehyde and ω,3,4,5-tetraacetoxyacetophenone under acidic conditions using ethyl acetate–ethanol containing hydrogen chloride. This is another example of the greater stability of phenolic benzoates as compared to acetates.

Testing of cannabidiol acid (CBDA) diacetate, furnished by Dr. Schultz, indicated that, as in the case of resorcinol diacetate, esterification was at least partly responsible in inhibiting the color reaction. Since the pure sample of CBDA was not available, the chromogenic activity of the free phenolic compound could not be determined to elucidate the effect of addition of carbonyl group in CBD molecule. In the paper chromatography of Lebanese hashish extract reported above, a blue spot, which appeared to be due to the presence of CBDA, was observed. The relative weak color of cannabidiol bis-3,5-dinitrobenzoate can also be rationalized as being due to the blocking of the phenolic hydroxyls by esterification and stability of the ester under the test conditions. Further identification and other studies on chromatography of *Cannabis* phenols are continuing.

Chromogenic Activity of Carbonyl-Substituted Resorcinol Derivatives.—In a previous communication (14) the results of color reactions of carbonyl-substituted resorcinols were reported. Phenols substituted with various electron-withdrawing carbonyl groups and hydroxyl groups in 2,4-, 2,6-, and 3,5-positions were tested. Results revealed a varying chromogenic activity of these three types of resorcinols. 2,6-Dihydroxycarbonyl-containing phenols showed the most sensitive color reaction, and the corresponding 3,5-dihydroxy compounds showed the least. In addition, testing of 5,7-dihydroxyflavans and related compounds revealed that our reagent was useful to identify these compounds and distinguish them from related compounds that have a carbonyl group in the 4-position in the flavan nucleus.

[4] Chromatography of *Cannabis* extract was performed at the Organic Chemistry and Narcotic Section, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada. The results were kindly provided by Dr. C. G. Farmilo, to whom the authors are indebted.

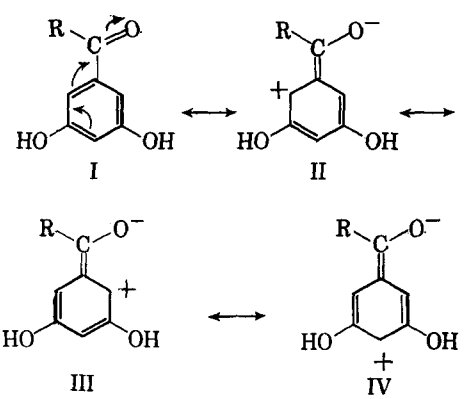
The authors have also examined the effect of an electron-donating group in mitigating the adverse effect of the electron-withdrawing carbonyl group. Color reactions of trihydroxyphenols containing the carbonyl group reported here provide influential data for additional hydroxyl in carbonyl-substituted resorcinols. Examination reveals weak color tests for carbonyl-containing pyrogallols and hydroxyhydroquinones measured against sensitive color reactions for similar phloroglucinols, an indication that the location of an additional hydroxyl group in the resorcinol molecule is important in mitigating the adverse effect of the carbonyl group.

The sensitive color reaction of *o*-desaspidinol (2,4-dihydroxy-6-methoxybutyrophenone) shows the effect of the electron-donating methoxyl group in overcoming the deactivation of the carbonyl group in resorcinol. Positive results of *o*-orsellinic acid (2,4-dihydroxy-6-methylbenzoic acid) and *o*-orcylaldehyde (2,4-dihydroxy-6-methylbenzaldehyde) indicate the influence of an electron-donating methyl group to compensate for the deactivating effect of the carbonyl group.

To explain the varying chromogenic activity of the three types of carbonyl-substituted resorcinols and relative sensitive color reactions of the carbonyl-substituted phloroglucinols, *o*-desaspidinol, *o*-orsellinic acid, and *o*-orcylaldehyde, the following explanation may be considered.

It is known that essentially all *ortho* substituents boost the acidity of benzoic acids. Gould (27) explains this as being due to the steric inhibition of resonance. Resonance effects with a conjugated system are most pronounced when the atoms of such a system lie in a common plane; such effects fall off rapidly as departures from planarity increase. Thus, according to Gould (27), *ortho* substituents in benzoic acids "will get in the way" of the carbonyl oxygen unless the latter move out of the plane of the benzene ring; when this happens, the acid-weakening resonance effect is diminished greatly (due to the nonplanarity).

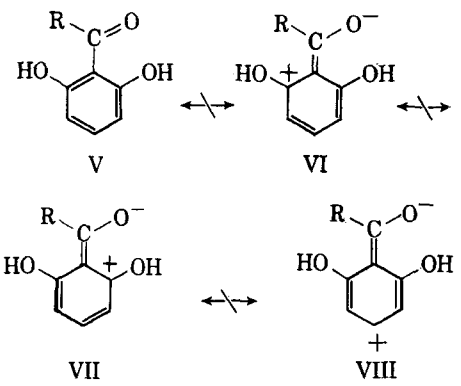
In considering the role of resonance effect in the carbonyl-substituted resorcinols and other related phenols tested, the statement generally can be made that electron withdrawal by the carbonyl group in conjugation with the ring would yield a positive charge on the *ortho* and *para* positions. Resonance effect would be possible in the 3,5-dihydroxycarbonyl compound and would cause deactivation of the ring, as shown in I–IV.[5] The deactivation would be



[5] In the carbonyl-substituted resorcinols tested, R was either H, CH_3, OH, or NH_2 (14).

quite pronounced in these compounds and might cause inhibition of color reaction.

In 2,6-dihydroxycarbonyl compounds, *o*-hydroxyl groups might bring about steric inhibition of resonance, as shown in V to VIII.



V VI VII VIII

The deactivation of the ring might be considerably less; 2,6-dihydroxycarbonyl compounds may react relatively easily with PDAB to yield colored products.

Reported results (14) of 2,4-dihydroxycarbonyl-substituted compounds indicate that the chromogenic activity of these compounds is higher than 3,5- and lower than 2,6-dihydroxycarbonyl-containing derivatives. On the other hand, 2,4-dihydroxycarbonyl-substituted compounds would react like 2,6-derivatives, probably due to steric inhibition of resonance if the 6-position in such compounds was substituted. The positive color reaction of carbonyl-substituted phloroglucinols, *o*-desaspidinol, *o*-orsellinic acid, and *o*-orcylaldehyde, in relatively low amounts indicate that this indeed is the case.

In conclusion, it should be pointed out that, at this stage in the investigation, no mechanisms of the reactions involved in the formation of colored products have been studied. Besides, most colors recorded were the authors' visual observations and may be subject to personal judgment. Additional study would enlighten the determination of the causative factors and confirm or disprove the suggested interpretations.

SUMMARY AND CONCLUSIONS

A simple paper spotting technique was employed to study the structure-chromogenic activity relationship of phenolic compounds with a modified Ehrlich reagent. Generally, six different amounts of over 100 phenols and derived compounds were tested in amounts varying from 1 to 100 mcg. Of these, 90 have been reported here.

Results of tests performed on mono-, di-, and trihydroxyphenols indicated that many phenols gave color reactions with the reagent. Among these, sensitive reactions were observed with resorcinol, naphthoresorcinol, phloroglucinol, pyrogallol, and hydroxyhydroquinone. Phloroglucinol yielded the most sensitive color test.

In considering resorcinol as the parent compound, the effect of various substituents on the chromogenic test had been examined. A study of several 4-alkyl-substituted resorcinols indicated that, with an increase in alkyl chain, an inhibition of color reaction occurred. Blocking of the phenolic hydroxyl group(s) by etherification and esterification resulted either in elimination or diminution of the chromogenic activity. The inhibition of color formation due to etherification was utilized in the identification of cannabidiol, the phenolic constituent of *C. sativa*. Alteration in the color reaction was, however, observed on testing aged and nonstored samples of cannabidiol.

Variation of the chromogenic activity of three types (2,4-, 2,6-, and 3,5-dihydroxy-) of carbonyl-substituted resorcinols previously reported (14) was studied further. The effect of electron donators, such as the hydroxyl, methoxyl, and methyl groups, in mitigating the adverse effect of carbonyl group in resorcinols was examined. With the 2,4-dihydroxycarbonyl group containing resorcinols, results appeared to indicate that any of these groups could overcome effectively the inhibition of the color reaction when substituted on the 6-position.

The testing of some naturally occurring phenols revealed that the modified Ehrlich reagent was a useful chromogen and may be applicable as a differentiating agent for compounds such as sesamol, cannabidiol, phloretin, orsellinic acid, and brazilin.

REFERENCES

(1) Ehrlich, P., *Med. Woche*, **1911**, 151; through Morton, A. A., "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 68.
(2) Feigl, F., "Spot Tests in Organic Analysis," 6th English ed., Elsevier Publishing Co., Amsterdam and New York, 1960, p. 289.
(3) Block, R. J., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," 2nd ed., Academic Press Inc., New York, N. Y., 1958, p. 318.
(4) Racusen, D., *J. Chem. Educ.*, **39**, 484(1962).
(5) Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry," 13th ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1954, p. 444.
(6) Morton, A. A., "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 68.
(7) Kapadia, G. J., *et al.*, *Lloydia*, **26**, 205(1963).
(8) Harada, T., and Nikumi, Z., *J. Agr. Chem. Soc. Japan*, **23**, 415(1950); through *Chem. Abstr.*, **45**, 10579 (1951).
(9) Acheson, R. M., Paul, R. M., and Tomlison, R. V., *Can. J. Biochem. Physiol.*, **36**, 295(1958).
(10) Shaw, K. N. F., and Thavarthen, J., *Nature*, **182**, 797(1958).
(11) Steelink, C., *ibid.*, **184**, 720(1959).
(12) McGreer, E. G., Robertson, M. C., and McGeer, P. L., *Can. J. Biochem. Physiol.*, **39**, 605(1961).
(13) Acheson, R. M., and Turner, I., *J. Chromatog.*, **7**, 520(1962).
(14) Kapadia, G. J., and Zalucky, T. B., *ibid.*, **15**, 76(1964).
(15) Block, R. J., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," 2nd ed., Academic Press Inc., New York, N. Y., 1958, pp. 102, 130, 137, 362, and 398.
(16) Reddi, K. K., and Kodicek, E., *Biochem. J.*, **53**, 286 (1953).
(17) Kornerup, A., and Wanscher, J. H., "Reinhold Color Atlas," Reinhold Publishing Corp., New York, N. Y., 1962.
(18) Korte, F., and Sieper, H., *Ann.*, **630**, 71(1960); personal communication.
(19) Kapadia, G. J., *et al.*, *J. Chromatog.*, **12**, 420(1963).
(20) Korte, F., and Sieper, H., *Tetrahedron*, **10**, 153(1960).
(21) Davis, T. W. M., Farmilo, C. G., and Osadchuk, M., *Anal. Chem.*, **35**, 751(1963).
(22) Mechoulam, R., and Shvo, Y., *Tetrahedron*, **19**, 2073(1963).
(23) Gaoni, Y., and Mechoulam, R., *J. Am. Chem. Soc.*, **86**, 1646(1964).
(24) Mechoulam, R., *J. Org. Chem.*, in press.
(25) Bergmann, M., and Dangschat, P., *Ber.*, **52**, 382 (1919).
(26) Bradley, W., Robinson, R., and Schwarzenbach, G., *J. Chem. Soc.*, **1930**, 793.
(27) Gould, E. S., "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, Inc. New York, N. Y., 1959, p. 236.