Laxatives II

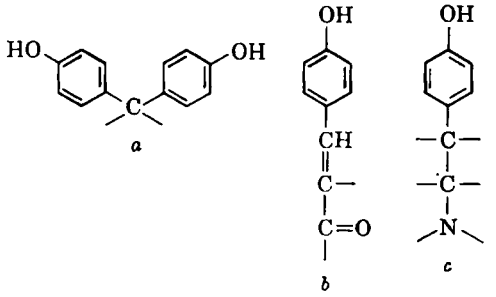
Relationship Between Structure and Potency

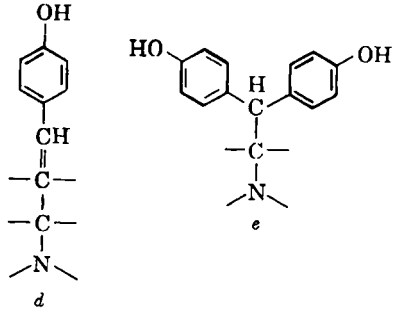
By MAX H. HUBACHER and SIDNEY DOERNBERG

The Relative Laxative Potency of a series of compounds was determined. These were mainly phenols containing certain groupings. We find two phenolic HO-groups, preferably in the configuration (*p*) HO-C_6H_4-C-C_6H_4-OH (*p'*) in the synthetic organic laxatives most commonly employed commercially. The two exhibiting the highest potency also contained a nitrogen ring. The Macaque monkey (*Macaca mulatta* and *M. speciosa*) gave the most consistent and meaningful potency values, data which could be applied successfully to humans in laxative action.

IN A PREVIOUSLY published paper on this subject (1), the observation was recorded that of 36 compounds tested, only those which had at least two phenolic hydroxyl groups had laxative properties, preferably those with the grouping *a* in their molecule. In the present work, this concept was explored further with the preparation and testing of additional compounds.

Included in this investigation were three phthaleins—an isomer of *o*-(4,4'-dihydroxybenzhydryl) benzyl alcohol, the latter previously found to be an excellent laxative (1); a mono-, di-, and tetrahydric alcohol; commercially available sennoside (2), which is a mixture of the naturally occurring glucosides extracted from senna; and other compounds having either of the groupings *a*, *b*, *c*, *d*, or *e* in their molecule.





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Methods of preparation are given for new compounds or for known compounds when the method either gives a better product or a better yield than the published one.

LAXATIVE POTENCY

The laxative potency of these compounds was determined on monkeys, as previously described (1). Until recently, the rhesus monkey (*Macaca mulatta*) was used exclusively because of the close similarity to human reaction to laxatives. Recently, Kling and Orbach (3), also Hensley and Richmond (4), described their experiences with a subspecies, *Macaca speciosa* or stump-tailed monkey. On the basis of their findings regarding the anatomical and behavioral characteristics of this species, it was decided to determine their suitability as a subject in laxative potency tests.

Results show that this animal makes an excellent subject and compares favorably with rhesus monkeys. Their response to laxatives is consistent, they take their doses willingly, are more docile and more resistant to infection than the rhesus monkey.

The laxative potency was determined by the equieffective dose method, compared to the same dose of a phenolphthalein N.F. XI, whose purity and laxative response had been determined on animals and humans. The doses were administered in food, mixed with cereal, or placed in wedges of apple, banana, melon, etc. The bottom of the cages are provided with a raised grid with 0.5-in. mesh opening, so that the soft stools are deposited on the supporting tray and can be easily counted. Three consecutive days of hard stools must intervene between administration of doses. If the initial dose produces no soft stools, it is increased by 1 mg./Kg. increments at 3-day intervals until a response of from two to five soft stools over a 3-day period is obtained. This dose is a direct relationship to the Unit Standard Dose for that animal and the Relative Laxative Potency (R.L.P.) is expressed in units obtained by the formula

$$\text{R.L.P.} = \frac{\text{Minimum Laxative Dose of Standard in mg./Kg.}}{\text{Minimum Laxative Dose of Unknown in mg./Kg.}}$$

This method is somewhat modified from previously published procedures (5–7) and has given consistent and comparable results.

For our purpose, a compound whose R.L.P. was

less than 0.1, *i.e.*, if a dose 10 times the minimum effective dose of phenolphthalein N.F. XI did not produce laxative effect, the compound was inactive for practical purposes.

EXPERIMENTAL[1]

Preparation of Compounds

Phenolthymolphthalein or 3-(4-Hydroxyphenyl)-3-(2-methyl-4-hydroxy-5-isopropyyphenyl)phthalide (I).—A mixture of 60.5 Gm. (0.4 mole) of thymol,[2] 48.4 Gm. (0.2 mole) of 2-(4-hydroxybenzoyl)benzoic acid (10), 20 Gm. of anhydrous zinc chloride, and 0.16 ml. of concentrated sulfuric acid was stirred for 2 hours at 120°. Dilute hydrochloric acid then was added and the unreacted thymol was steamed out (28 Gm.). The crude phthalein (70.5 Gm., m.p. 270–275°) was dissolved in 1600 ml. of boiling ethanol. On cooling, four crops of crystals totaling 67.8 Gm. (90%), m.p. 284–286°, were obtained.

The pure phthalein (I) formed colorless needles, melting at 285–286° [Ghatak (11) gives a m.p. of 231–232° and Kin (12) gives a m.p. of 276° for this phthalein]; it dissolved in normal sodium hydroxide with a deep purple color.

Anal.—Calcd. for $C_{24}H_{22}O_4$: C, 76.98; H, 5.92. Found: C, 77.14; H, 6.27.

Subjecting 7.5 Gm. of I to the Friedländer oxime splitting (10), yielded 1.4 Gm. of 2-(4-hydroxybenzoyl)benzoic acid and 1.1 Gm. of an amine, m.p. 177–179°, which was identical with 2-isopropyl-4-amino-5-methylphenol (13). This finding proves that both HO-groups in I are in the *para* position to the center carbon atom of the triphenylmethane molecule.

Diacetylderivative of I.—This compound, recrystallized from ethanol, formed colorless crystals melting at 156.3 to 157.1°.

Anal.—Calcd. for $C_{28}H_{26}O_6$: C, 73.36; H, 5.67; mol. wt. 458. Found: C, 73.28; H, 5.37; mol. wt. 480.

The Dimethylether of I.—This compound, prepared from I by either the methyl iodide or the dimethyl sulfate method, after crystallization from 41% ethanol (1 Gm. in 240 ml.) formed colorless, fine needles, melting at 141.3 to 142.1° [Kin (12) reported a m.p. of 122°].

Anal.—Calcd. for $C_{26}H_{26}O_4$: C, 77.58; H, 6.51. Found: C, 77.20; H, 6.22.

Phenolcatecholphthalein or 3-(p-Hydroxyphenyl)-3-(m,p-dihydroxyphenyl)phthalide (II).—This phthalein was not easily purified since it did not crystallize readily. Therefore, patience was needed to obtain seed crystals.

The following procedure gave a good product in fair yields. A mixture of 22.0 Gm. (0.2 mole) of catechol, 48.4 Gm. (0.2 mole) of 2-(4-hydroxybenzoyl)benzoic acid (10), 20 Gm. anhydrous zinc chloride, and 0.1 ml. of concentrated sulfuric acid were stirred for 2 hours at 120°. To remove the dark, sticky mass from the flask, it was refluxed with 200 ml. of ethanol until dissolved, requiring 1 to 2 hours. The solution was poured into 1 L. of water. The precipitated, pliable mass became hard after standing for several days (56–61 Gm., m.p. 192–198°). It was crystallized from 50% acetic acid (1 Gm. in 2 ml.) or from 20% ethanol (1 Gm. in 20 ml.), stirring the solution while cooling at a rate of 1° an hour; 45–50 Gm. (67–75%), m.p. 203–206°, was obtained.

Sometimes, even after seeding, no crystals could be obtained. In such cases, the amorphous mass was acetylated and the purified triacetyl derivative hydrolyzed as described below.

The pure phenolcatecholphthalein (II) melted at 203.5–208.0° [Ghatak (11) recorded a m.p. of 148–149°, while Kin (12) could not obtain it in crystalline form].

Anal.—Calcd. for $C_{20}H_{14}O_5$: C, 71.85; H, 4.22; mol. wt. 334. Found: C, 71.48; H, 4.46; mol. wt. 339.

The dark reddish-brown solution of II in 1 *N* sodium hydroxide turned brown on exposure to air at 25° for several weeks, and 2-(4-hydroxybenzoyl)-benzoic acid was isolated from the solution.

The trimethylether of II was obtained as a non-crystallizable oil.

Triacetylderivative of II.—This compound was prepared from II, acetic anhydride, and a trace of sulfuric acid. After crystallizations from ethanol (1 Gm. in 16 ml.), it melted at 154.5 to 155.6° [Kin (12) recorded a m.p. of 148°].

Anal.—Calcd. for $C_{26}H_{20}O_8$: C, 67.82; H, 4.38; mol. wt. 460. Found: C, 67.45; H, 4.73; mol. wt. 451.

This triacetylderivative was best hydrolyzed by refluxing for 1.5 hours a solution of 6 Gm. in 30 ml. of ethanol and 0.05 ml. of sulfuric acid. The solvent was evaporated *in vacuo* and 100 ml. of water was added to the residue. The amorphous precipitate of II slowly became crystalline.

Alkaline hydrolysis is not recommended because of the instability of II in alkaline solutions.

Phenoltoluenephthalein or 3-(p-Hydroxyphenyl)-3-(p-tolyl)phthalide III.—A mixture of 48.0 Gm. (0.2 mole) of *o*-(*p*-toluoyl)benzoic acid (14), 28.2 Gm. (0.3 mole or 50% excess) of phenol, 20 Gm. anhydrous zinc chloride, and 0.5 ml. concentrated sulfuric acid were stirred for 8.5 hours at 120–125°. The hot, viscous reaction mass was poured into 1 L. of water, removing the part adhering to the flask with hot ethanol. The crude phthalein was washed five times with hot water, cooling and decanting the water each time. The phthalein (60–65 Gm., m.p. 121–140°), which now had become hard, was crystallized from acetic acid (1 Gm. in 2.5 ml.). If no seed crystals are available, then crystallization may not set in for many weeks. Two crops of crystals, totaling 37.3 to 41.8 Gm. (59–66%), m.p. 149–153°, were obtained.

This phthalein may also be crystallized from benzene (1 Gm. in 8 ml., recovery 93%), from which solvent it crystallizes with 1 mole of solvate benzene, which it loses slowly at 25°, rapidly at 120°. Calcd. for $C_{21}H_{16}O_3 \cdot C_6H_6$: benzene 19.8%; loss at 120°: 20.4 ± 1.1%.

The pure III melts at 154.1 to 155.9°.

Anal.—Calcd. for $C_{21}H_{16}O_3$: C, 79.73; H, 5.10; mol. wt. 316. Found: C, 79.70; H, 5.90; mol. wt. 319.

A solution of III in 5 *N* sodium hydroxide is colorless, whereas a solution in sulfuric acid is orange. III is very soluble in acetone, ethanol, *n*-butanol, cyclohexanone, and in chloroform.

[1] All melting points are corrected. Molecular weights determined by the Signer method as described by Clark (8). Color designations are by the Munsell color system (9).

[2] This is an excess of 100% over theory, the excess acting as a solvent.

TABLE I.—COMPOUNDS AND THEIR LAXATIVE POTENCY

No.	Name	Contains Group	Prepn.	M.p., °C. cor.	Potency (N.F. Phenolphthalein = 1)
I	Phenolthymolphthalein	...	Described in text	285–286	Inactive
II	Phenolcatecholphthalein	...		203.5 to 208.0	1.4
III	Phenoltoluenephthalein	...		154.1 to 155.9	2.0
IV	*o*-(4-Hydroxy-4′-methylbenzhydryl) benzoic acid	...		221.7 to 222.5	Inactive
V	Pyromellityl alcohol	...		191.2 to 191.8	Inactive
VI	3,6-Dihydroxy-9-xanthone	...		>320	Inactive
VII	1,1-Bis(*p*-hydroxyphenyl)-cyclohexane	*a*		187.8 to 188.9	Inactive
VIII	2-Phenyl-4-(*p*-hydroxybenzylidene)-5-oxazolone	*c*		221.5 to 221.9	Inactive
IX	*p*-Hydroxybenzylidene-hydrazide of the bis(*p*-hydroxyphenyl)acetic acid	*a*, *e*		262.5 to 263 0	Inactive
X	2-(*p*-Hydroxystyryl)pyridine	*d*		216.4 to 217.4	Inactive
XI	2,6-Bis(*p*-hydroxystyryl)-pyridine	*d*		258.5 to 259.0	Inactive
XII	2-(*p*-Hydroxyphenyl)-1,3-indandione	...		177.1 to 178.1	Inactive
XIII	*o*-(2,4-Dihydroxybenzhydryl)-benzoic acid	...	Hubacher, M. H., *J. Org. Chem.*, **23**, 1402 (1958).	186.9 to 187.4	Inactive
XIV	*o*-(2,4-Dihydroxybenzhydryl) benzyl alcohol	...	*Ibid.*	169.8 to 170.4	2.1
XV	Ricinoleyl alcohol	...	Marketed as Aldol by Archer-Daniels-Midland Co., Cleveland, Ohio	Colorless oi	Inactive
XVI	Phthalyl alcohol	...	Nystrom, R. F., and Brown, W. G., *J. Am. Chem. Soc.*, **69**, 1198(1947).	63.6 to 64.1	Inactive
XVII	Sennosides Sandoz	...	A mixture of equal parts Sennoside A and B (2)	220–250	6.1
XVIII	10,10-Bis(*p*-hydroxyphenyl)-9-anthrone	*a*	Blicke, F. F., and Patelski, R. A., *J. Am. Chem. Soc.*, **60**, 2636(1938).	311–314	Inactive
XIX	4,4′-Dihydroxybenzil	...	Hubacher, M. H., *J. Org. Chem.*, **24**, 1950 (1959).	250.2 to 251.4	Inactive
XX	Ethyl bis(*p*-hydroxyphenyl)-acetate	*a*	*Ibid.*	163.8 to 165.7	Inactive
XXI	Hydrazide of the bis(*p*-hydroxyphenyl)acetic acid	*a*, *e*	Nathansohn, G., *ibid.*, **26**, 1421(1961).	252.0 to 253.6	Inactive
XXII	4,4′-Dihydroxytriphenyl-acetic acid	*a*	*J. Med. Chem.*, **7**, 571(1964).	257–259 dec.	Inactive
XXIII	4,4′-Dihydroxytetraphenyl-methane	*a*	Gomberg, M., and Jickling, R. L., *J. Am. Chem. Soc.*, **37**, 2580(1915).	294–296	Inactive
XXIV	1,5-Bis(*p*-hydroxyphenyl)-1,4-pentadiene-3-one	*b*	Zincke, T., and Mühlhausen, G., *Ber.*, **36**, 131(1903).	245.6 to 246.1	Inactive
XXV	4,4′-Dihydroxychalcone	*b*	Vorländer, D., *ibid.*, **58**, 128(1925).	201.2 to 202.1	Inactive
XXVI	2-(*p*-Hydroxybenzylidene)-1-indanone	*b*	Feuerstein, W., *ibid.*, **34**, 413(1901).	222.3 to 224.6	Inactive
XXVII	2-(*p*-Hydroxybenzylidene)-1,3-indandione	*b*	v. Kostanecki, S., and Laczkowski, *ibid*, **30**, 2141(1897).	240.8 to 242.2	Inactive
XXVIII	2-(*p*-Hydroxybenzoyl)-1,3-indandione	...	Horton, R. L., and Murdock, K. C., *J. Org. Chem.*, **25**, 940(1960).	280–283 dec.	Inactive
XXIX	α-(*p*-Hydroxybenzylidene)-hippuric acid	*c*	Erlenmeyer, E., Jr., and Halsey, J. T., *Ann.*, **307**, 140(1899).	238–240 dec.	Inactive
XXX	3-Phenyl-4-(*p*-hydroxybenzylidene)-5(4)-isoxazolone	*b*, *d*	Wahl, A., and Meyer, A., *Compt. Rend.*, **146**, 639(1908).	213.0 to 213.5	Inactive
XXXI	2,4-Diamino-6(*p*-hydroxystyryl)-*s*-triazine sulfate	*d*	v. Humnicki, *Zentralbl., II*, 706(1907).	290	Inactive
XXXII	di(*p*-Hydroxyphenyl)-(2-pyridyl) methane	*a*, *c*	Karl Thomae, G.m.b.H., Brit. pat. 730,-243; through *Chem. Abstr.*, **50**, 6515*f* (1956).	239–241	24

When 5 Gm. of III and 25 Gm. of KOH were heated in a small distilling flask for 5 minutes at 225°, toluene distilled off. From the cooled melt dissolved in water, 0.91 Gm. benzoic acid and 0.44 Gm. *p*-hydroxybenzoic acid were isolated.

Acetylderivative of III.—This compound, prepared from III and acetic anhydride, was obtained as an oil which became crystalline on rubbing with ethanol. It was purified further by dissolving it in absolute ether (1 Gm. in 10 ml.); on adding petroleum ether (10 ml.) to the solution, fine needles were obtained, which melted at 93–97°.

Anal.—Calcd. for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.17; H, 5.16.

Methylether of III.—This compound was made from III by either the dimethyl sulfate or methyl iodide method. It was crystallized from ethanol (1 Gm. in 2 ml.) or from absolute ether (1 Gm. in 8

ml.), formed rectangular plates, and melted at 94–99°.

Anal.—Calcd. for $C_{22}H_{18}O_3$: C, 79.94; H, 5.49; mol. wt. 330. Found: C, 80.32; H, 5.61; mol. wt. 357.

Phenoltoluenephthalin or o-(4-Hydroxy-4-methylbenzhydryl Benzoic Acid (IV).—To a solution of 15.8 Gm. of III in 60 ml. of 2.5 *N* sodium hydroxide, 5.0 Gm. of powdered Raney alloy (Ni-Al) was added over a period of 1 hour. The temperature of the reaction solution went up to 45–55°. The mixture was stirred for another 30 minutes, the nickel filtered off, and the filtrate acidified. The washed precipitate was redissolved at 25° in 110–140 ml. 2 *N* sodium carbonate, the aluminum hydroxide filtered off, and the filtrate acidified. The crude acid (15.0 to 15.8 Gm., m.p. 198–211°) was crystallized from 41% ethanol (1 Gm. in 20 ml.) or from 50% acetic acid (1 Gm. in 30 ml.). By sublimation at 190° and 10-μ pressure, and crystallizing the sublimate from 41% ethanol (1 Gm. in 31 ml.), a very pure acid (IV) was obtained, colorless needles melting at 221.7 to 222.5°. Its solution in dilute alkalies is colorless. It was very soluble in acetone and ethanol, soluble in acetic acid, and insoluble in water, benzene, or chloroform.

Anal.—Calcd. for $C_{21}H_{18}O_3$: C, 79.21; H, 5.70; mol. wt. 318. Found: C, 79.26; H, 5.78; neut. equiv. 315.

The Acetylderivative of IV.—3.18 Gm. of IV, 0.5 Gm. of anhydrous sodium acetate, and 5 ml. of acetic anhydride were heated for 1 hour to 120°. The compound was recrystallized from ethanol; crystals were also obtained by adding petroleum ether (6 ml.) to its solution in benzene (1 Gm. in 6 ml.). This acetylderivative formed colorless crystals melting at 179.7 to 181.0°.

Anal.—Calcd. for $C_{23}H_{20}O_4$: C, 76.64; H, 5.59. Found: C, 76.51; H, 5.08.

2-Methyl-9-acetoxy-19(p-acetoxyphenyl)anthracene (IVa).—When 0.05 ml. concentrated sulfuric acid was added to a suspension of 3.18 Gm. of IV in 5 ml. of acetic anhydride, the mixture became hot, and a dark green, clear solution was obtained. It was heated for a few minutes at 100°, cooled, and the crystal mush poured on ice. The greenish crude compound, after crystallization from ethanol (1 Gm. in 46 ml.) or from acetone, formed faint yellow crystals, m.p. 180.8 to 182.0°. Its solutions exhibited bluish fluorescence.

Anal.—Calcd. for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24. Found: C, 78.23; H, 5.44.

A suspension of 5.0 Gm. of IVa in 5 ml. of ethanol and 26 ml. of 2 *N* sodium hydroxide was refluxed for 2 hours. On acidification of the dark solution, an amorphous, dark precipitate was obtained (3.2 Gm.). Since it could not be made to crystallize, it was mixed with 20 Gm. zinc dust and heated in an atmosphere of hydrogen to 300°. The small quantity of sublimate, after several crystallizations from ethanol, formed needles of faint yellow, m.p. 175.3 to 176.1°.

Anal.—Calcd. for $C_{15}H_{10}O_2$: C, 81.08; H, 4.53. Found: C, 81.03; H, 4.66.

This compound was proved, by the mixed melting point method, to be 2-methylanthraquinone.

Pyromellityl Alcohol or 1,2,4,5-Tetrahydroxymethylbenzene (V).—The isolation of this alcohol is difficult because of its insolubility in organic solvents and its great solubility in water. Therefore, the specified quantities of water and acid must be used. Our method of isolation of this alcohol is simpler than that described by Benning and Grosskinsky (15).

The thimble of an extraction apparatus was charged with 12.4 Gm. (0.4 mole) of pyromellitic acid tetramethyl ester (m.p. 141–142°) and the flask contained a solution of 9.2 Gm. $LiAlH_4$ in 400 ml. of absolute ether. After the thimble content was dissolved, refluxing was continued for an additional 20 hours. Then 160 ml. of water was added dropwise, at the same time distilling out the ether, and finally 90 ml. of 10 *N* sulfuric acid to dissolve the aluminum hydroxide. The warm solution was cooled overnight to room temperature, and the crystals which had formed were filtered off and washed with a little ice water.

The dried compound (6.7 to 8.3 Gm.) was purified by extracting it in a thimble extractor with 60 ml. of methanol. The crystals formed in the solvent weighed 6.5 to 6.9 Gm. (80–87%), m.p. 190–191°.

This alcohol also could be crystallized from water (1 Gm. in 5.4 ml.) or sublimed at 195° and 6-μ pressure. It melted at 191.2 to 191.8° [literature 191–192° (15)].

3,6-Dihydroxy-9-xanthone (VI).—A slurry of 3.0 Gm. of 2,2′,4,4′-tetrahydroxybenzophenone (m.p. 197–200°) in 30 ml. of water was heated at 220–230° in a sealed tube for 14 hours. The cooled mixture contained long needles (2.62 Gm.) which when crystallized from a considerable quantity of 20% ethanol, formed fine silky needles which did not melt even at 320°. Its alkaline solutions exhibited strong bluish fluorescence. Its diacetylderivative melted at 206.5 to 207.0° (from ethanol) [literature 204° (16)].

1,1-Bis(p-hydroxyphenyl)cyclohexane (VII).—This compound was prepared in 20% yield by condensing phenol with cyclohexanone in the presence of concentrated sulfuric acid. After crystallizations from 1,2-dichloroethane (1 Gm. in 17 ml.) and drying at 120°, VII melted at 183°, then solidified and melted again at 187.8 to 188.9° [literature 186° (17)].

2-Phenyl-4-(p-hydroxybenzylidene)oxazol-5-one (VIII).—This compound has been made in low yields either by demethylation of its methyl-ether or by heating (*p*-hydroxybenzylidene)hippuric acid at 200° (18).

The following method produced pure VIII in high yields: (*p*-hydroxybenzylidene)hippuric acid (14.15 Gm., m.p. 238° dec.) (19) and 25 ml. of thionyl chloride were heated slowly to 85° until evolution of HCl ceased. The excess $SOCl_2$ was distilled off *in vacuo*. The yellow cake was treated with 50 ml. of benzene and filtered: 10.7 to 11.1 Gm. (80–85%) VIII, m.p. 215–218°, was obtained.

After crystallization from 1,4-dichloroethane (1 Gm. in 50 ml.) or sublimation at 200° and 6μ pressure, VIII was obtained as crystals of strong yellow color, melting at 221.3 to 221.9°. Its solution in 0.1 *N* sodium hydroxide was unstable at room temperature. The initially strong orange of the solution soon faded and on acidification, (*p*-hydroxybenzylidene)hippuric acid precipitated.

Propionylderivative of VIII.—This compound made from VIII, propionic acid, and a drop of con-

centrated sulfuric anhydride, after crystallization from considerable ethanol, formed fine, light yellow needles melting at 161.2 to 161.8°.

Anal.—Calcd. for $C_{19}H_{15}O_4N$: Theory: C, 71.02; H, 4.70; N, 4.35. Found: C, 70.92; H, 4.51; N, 4.83.

p-Hydroxybenzylidenehydrazide of the Bis(p-hydroxyphenyl) Acetic Acid (IX).—A solution of 2.53 Gm. of the hydrazide of the bis(*p*-hydroxyphenyl)acetic acid (20) (m.p. 252.1 to 253.1°) and 1.22 Gm. *p*-hydroxybenzaldehyde in 25 ml. of 41% ethanol were refluxed for 2 hours. The fine needles (3.57 Gm.) were crystallized from 20% ethanol (1 Gm. in 35 ml.). Pure IX sintered at 215–220°, loosing thereby 5.0% water (theory for $C_{21}H_{18}N_2O_4 \cdot H_2O$ = 4.7% H_2O), then melted at 262.5 to 263.0°.

Anal. (of compound dried at 220° *in vacuo*)—Calcd. for $C_{21}H_{18}N_2O_4$: C, 69.61; H, 4.97; N, 7.73. Found: C, 69.70; H, 5.13; N, 7.40.

2-(4-Hydroxystyryl)pyridine or 4'-Hydroxystilbazole (X).—A solution of 18.6 Gm. (0.2 mole) of α-picoline, 24.4 Gm. (0.2 mole) of *p*-hydroxybenzaldehyde, and 41 Gm. (0.4 mole) of acetic anhydride were refluxed for 12 hours (153–160°). The unreacted picoline and acetic acid were distilled off *in vacuo*. After adding 150 ml. of 3 *N* HCl and refluxing for 1 hour, water was added and the solution adjusted to pH 7. The greenish-gray precipitate weighed 21.7 Gm. (55%) and melted at 197–207°.

Crystallized from ethanol (1 Gm. in 18 ml.) and then sublimed at 200° and 6-μ pressure, X melted at 216.4 to 217.4° [literature 219–220° (21)]. The compound had a faint yellow tinge, dissolved in 0.1 *N* NaOH with a light yellow and in 0.1 *N* HCl with a light greenish color.

Acetylderivative of X.—This was prepared from X, acetic anhydride, and a trace of sulfuric acid. It was crystallized by adding petroleum ether to its saturated benzene solution. It melted at 109.2 to 110.3°.

Anal.—Calcd. for $C_{15}H_{12}O_2N$: C, 75.31; H, 5.43; N, 5.85. Found: C, 75.67; H, 5.35; N, 6.02.

2,6-Bis(p-hydroxystyryl)pyridine (XI).—A mixture of 2,6-bis(*p*-acetoxystyryl)pyridine (30.0 Gm.; m.p. 183–184°) (21) in 90 ml. of acetic acid, 30 ml. of water, and 3 ml. of sulfuric acid was kept for 12 hours at 110–120°. The yellow compound, after crystallization from isopropanol, melted at 258.5 to 259.0°, formed yellowish-orange crystals and dissolved in aqueous alkali with a strong green color. For analysis, it was dried at 150° in high vacuum.

Anal.—Calcd. for $C_{21}H_{17}O_2N$: C, 80.00; H, 5.39; N, 4.44. Found: C, 79.14; H, 5.61; N, 4.43.

2-(p-Hydroxyphenyl)-1,3-indandione (XII).—A 10.0-Gm. quantity of 2-(*p*-anisyl)-1,3-indandione [m.p. 154–155° (22)] and 20.0 Gm. of pyridine hydrochloride were heated for 2 hours at 180°. The cooled melt was dissolved in water, the solid filtered off, washed, dried (9.2 to 9.4 Gm.), and crystallized from 1,2-dichloroethane (1 Gm. in 20 ml.). The compound formed faint yellowish needles, which melted at 177.1 to 178.1° to a red melt. The color of its solution in 0.1 *N* sodium hydroxide was dusky red. The crystals contained solvate solvent which they lost at 100°.

Anal.—Calcd. for $C_{15}H_{10}O_3 \cdot \frac{1}{2}\ ClCH_2CH_2Cl$: Dichloroethane 17.2. Found: loss of weight at 100° = 17.1. A sample dried at 100° *in vacuo* was analyzed: $C_{15}H_{10}O_3$: C, 75.62; H, 4.20; mol. wt. 238. Found: C, 75.13; H, 4.19; mol. wt. 248.

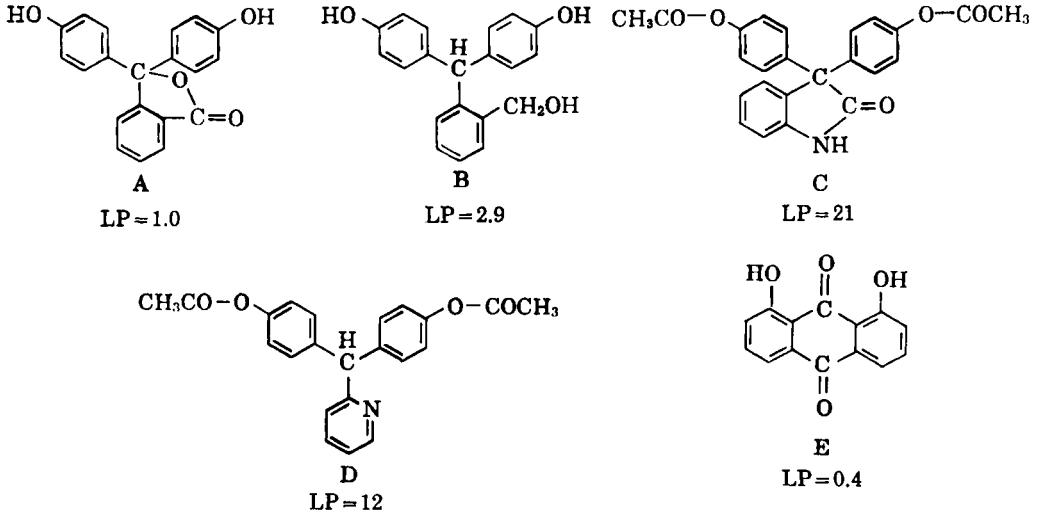
DISCUSSION

Many compounds prepared and found to be inactive, namely VII, VIII, XVIII, XXIII, and XXIV, were reported by Schmidt and Seeger (23) to be active when tested on rats in rather huge doses. Unfortunately, many compounds in the literature described as laxatives, actually are inactive in humans. Therefore, in evaluating these substances, it must be emphasized again that the test animals and dosages must be considered.

The distinct laxative action of sennosides, the principal active ingredient isolated in pure form from senna (2), was confirmed as being six times more potent than phenolphthalein.

None of the ten compounds having either grouping *b*, *c*, or *d* in their molecule was active.

The two phthaleins, II and III, possess laxative properties. It was surprising that III, which has only one HO-group, was active.



Scheme I

Of the four mono- and polyhydric alcohols tested, namely V, XIV, XV, and XVI, only the one which also has two phenol hydroxyls (XIV) showed distinct laxative properties. It is noteworthy that its position isomer, compound B in Scheme I, had previously been found to be a good laxative (1). These findings indicate that the phenolic HO-group is of greater importance than the alcohol hydroxyl, and that new laxatives might be found among the diphenolic benzyl alcohols.

Scheme I shows the structural formulas of the five synthetic organic compounds which are being used presently as active ingredients in commercial laxatives:

Phenolphthalein (*A*).—This is the most widely used of the five, is odorless, tasteless, and practically devoid of untoward effects. Studies on various animals have attested repeatedly to its low order of toxicity, all investigators having failed to establish LD_{50} (24–26).

Phenolphthalol (*B*) (1, 27).—This is used mainly in West Germany under the name of Egmol, and likewise is nontoxic (26).

3,3-Bis(p-acetoxyphenyl)oxindole (*C*) (28).—This compound is known as Isacen and is the most potent of all five. Baum *et al.* (29) claimed to have detected "diphenylisatin" or deacetylated compound C in canned prune juice. Neither we nor others were able to detect any 3,3-bis(*p*-hydroxyphenyl)oxindole in such juice. A private communication[3] states: "An exhaustive examination of prune juice failed to reveal the presence of isatin derivatives. Bioassays for the laxative effect of prune fractions have shown that the laxative action is in the fraction containing magnesium."

Bis(p-acetoxyphenyl)-(2-pyridyl)methane (*D*) (23, 27).—This is known as Dulcolax, the newest laxative, and is administered rectally as well as orally.

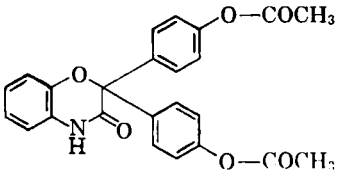
1,8-Dihydroxyanthraquinone (*E*) (30).—This compound is known under such names as chrysazin, Istin, Istizin, dantheon, or Dorbane, is related to the naturally occurring laxatives such as emodine, chrysophanic acid, and rhein, all of which have HO-groups in position 1 and 8 of anthraquinone.

It is interesting to note that compounds A, B, and C were first synthesized by A. Baeyer, but their therapeutic value was discovered much later by others.

Compounds A, B, C, and D contain group *a*, which has been called the laxaphoric group (31), and in addition, a third ring, benzene or pyridine, attached to the center C atom. Acetylation of the two HO-groups decreases the potency, exemplified by compounds C and D, which possess only half the potency of the parent compounds.

The two nitrogen containing compounds, C and D, are the most potent laxatives and contain group *e*. Recently, Schmidt and Seeger (32) synthesized several derivatives of 1,4-benzoxazoline with group *e* in their molecule. They found compound F to have approximately three times the potency of phenolphthalein when tested on rats.

[3] Letter dated July 18, 1963, from the Fruit and Pharmacology Laboratories, Western Regional Research Laboratory, Albany, Calif.



F

Our compounds with group *e*, IX and XXI, were inactive. Schraufstätter (33) synthesized four compounds with group *e* and found them all to be devoid of laxative properties.

Several other compounds with the laxaphoric group *a* are good laxatives, *i.e.*, 4,4′-dihydroxytriphenylmethane, the 1,8- and the 2,1-phenolnaphthaleins (1), and several compounds described by Schmidt and Seeger (23). However, the majority of compounds with group *a*, made and tested by us and others, were inactive.

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