were produced. Br and I produced a brown, tar-like deposit. The I also produced fumes of HI.

Of the elements of the fifth group, P, Sb and Bi were used. With Cl the P burst into flame immediately as stated in connection with copper. The bromine solution was decolorized the next morning, the I sol. was partly decolorized. Sb decolorized the Cl solution immediately, the Br solution was almost colorless the next morning, the I solution partly decolorized. Bi did not seem to react.

In several instances colorless crystals were obtained (NaCl, NaBr, $SbCl_3$), in other instances the bright surface of the metallic element was dulled (in the bromine experiment the Cu became black), in still other instances a tar-like product was deposited ($AlBr_3$).

It may be of interest to note that of the metallic elements sodium appeared more reactive than lithium, and cadmium more than zinc. On the other hand in Group five, P was much more reactive than Sb and Bi. Also, that, with the possible exception of Cu, the order of reactibility of the halogens proved to be Cl, Br, I, as was to be expected.

* For earlier reports see:

No. 1, JOUR. A. PH. A., 9, 857 (1920). No. 2, *Ibid.*, 9, 860 (1920). No. 3, *Ibid.*, 11, 1042, 1153 (1922). No. 4, *Ibid.*, 10, 26 (1921). No. 5, *Ibid.*, 11, 995 (1922).

(1) The principal object was the isolation and identification of the aldehydes. See P. A. Foote, JOUR. A. PH. A., 18, 350 (1929). After shaking out the aldehydes with aqueous sodium acid sulphite from the fractions with a higher boiling point than that of heptane, a complex mixture was obtained. This was fractionated by C. Sondern (*Thesis*, U-W. 1928). Of the fractions thus obtained those marked E. VII and VIII were employed.

(2) Fractions E. VII (b. p. 94.0–94.5°; d = 0.6800 at 25°) and E. VIII (b. p. 94.5–95.0°; d = 0.6815 at 25.5°) of Sondern, obtained by refractionating the portion of the oil distilling immediately above the boiling point of heptane, were used.

(3) Taylor and Hildebrand, *J. A. C. S.*, 45, 682 (1923).

(4) Hildebrand and Jenks, *Ibid.*, 42, 2180 (1920).

THERAPEUTIC SUBSTANCES DERIVED FROM UNSYMMETRICAL DIPHENYL COMPOUNDS III. SOME ARYL ESTERS OF THE HYDROXY DIPHENYLS.*

BY S. E. HARRIS AND W. G. CHRISTIANSEN.[1]

Numerous references have appeared in the literature describing the use of aryl esters of phenol and cresols as urinary and intestinal antiseptics. In extending our survey of diphenyl compounds it was decided to prepare a number of aryl esters of 2-, 3- and 4-hydroxy-diphenyls and some of their substitution products.

The three hydroxy diphenyls, (*o*-, *m*- and *p*-phenyl-phenol) and their alkyl and halogen substitution products are active, non-toxic germicides and it was hoped that by oral administration in the form of an ester they might reach the urine unmetabolized, and there exert the required germicidal action. Fifteen esters were prepared and tested. Results of animal experiments definitely show that they possessed no value as urinary antiseptics.

* Scientific Section, A. PH. A., Washington meeting, 1934.

[1] Research Department of the Chemical and Pharmaceutical Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.

The tests were carried out upon rabbits, to which the esters, dissolved in olive oil, at a dose level of 20 mg./Kg., were administered orally twice daily for five or six consecutive days. The urine was collected from the second day on and examined for germicidal activity, following in general the procedure of Leonard (1) except that the samples were diluted as required and plated for count immediately after culture, and after 24 hours' contact.

Early results led to the belief that some of the esters possessed activity, but it was later discovered that the urine of the animals varied in activity, and in many cases the urine of untreated controls was germicidal. The variations were correlated with the diet and p_H of the urine, and in later work the p_H was adjusted to 6.5–7.5 (quinhydrone electrode). The effect of p_H adjustment upon the germicidal results is shown in the following tables. 63 urine specimens were collected from rabbits which had been on a uniform diet of hay and oats for some time.

I. p_H Distribution

p_H	Below 6.5	6.5–7.5	Above 7.5
No. of urine specimens	23	16	24

II. Tested against *B. coli* and *Staphylococcus aureus*.

(*a*) p_H

	Below 6.5		6.5–7.5		Above 7.5	
	No. of Specimens Tested.	No. Showing Germicidal Activity.	No. of Specimens Tested.	No. Showing Germicidal Activity.	No. of Specimens Tested.	No. Showing Germicidal Activity.
B. Coli	21	8	14	1	22	13
Staph.	21	4	14	1	22	17

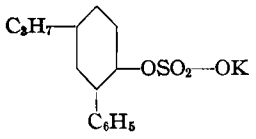
(*b*) p_H

	Below 6.0		Above 8.0	
	No. of Specimens Tested.	No. Showing Germicidal Activity.	No. of Specimens Tested.	No. Showing Germicidal Activity.
B. Coli	8	4	18	14
Staph.	8	3	18	16

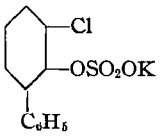
18 normal urine specimens with p_H below 6.5 or above 7.5 were then adjusted by NaOH or HCl to a value between 6.5 and 7.5. None of these adjusted samples showed any germicidal or antiseptic activity against the test organisms.

Further tests of the esters, in which the p_H of the urine samples was adjusted before test, confirmed the statement made above that the esters were not of value as urinary antiseptics.

Therapeutic activity was expected to result from liberation of the free phenol by hydrolysis of the esters in the body. Phenols, while sometimes excreted in part as glycuronates or even unchanged, are usually metabolized by the potassium salt of their acid sulphuric esters (2). These esters are stable in alkaline but not in acid solutions. The acid sulphuric esters of two of the most active phenols: Potassium 4-*n*-propyl-2-phenyl-phenyl sulphate (I) and Potassium 6-chloro-2-phenyl-phenyl sulphate (II) were prepared, tested *in vitro* and were found to be quite inactive.



I.



II.

In an alkaline or neutral urine such salts as these would represent the condition of phenol, whereas the free phenol would be expected in an acid urine. The absence of germicidal action in acid urines seems to indicate that the esters are not excreted after hydrolysis and combination with sulphuric acid, but that they pass through without material change.

In planning the work which has been outlined, we included one compound not of the diphenyl series: the benzoate of 5,7-dichloro-8-hydroxy-quinoline. It is here included for purposes of record.

EXPERIMENTAL.

The several compounds, with their corresponding crystallizing media, their melting points and analyses, are listed in the accompanying table. The esters were prepared by several methods, an example of each being detailed below. Excellent yields were obtained.

1. SCHOTTEN-BAUMANN METHODS.

3-Phenyl-Phenyl Benzoate.—42 Gm. *m*-phenyl-phenol, 43 Gm. benzoyl chloride and 50 cc. 35 per cent sodium hydroxide were mixed with 150 cc. water and shaken mechanically until the odor of benzoyl chloride had disappeared. The precipitated ester was filtered off, washed with water and dissolved in alcohol. Upon spontaneous evaporation of the alcohol the ester was deposited in fine crystals, m. p. 57–58° C. (corr.).

2. ACTION OF ACID CHLORIDE ON THE PHENOL IN A SOLVENT.

2-Phenyl-4-Chlorophenyl Benzoate.—40 Gm. 2-phenyl-4-chlorophenol and 30 Gm. benzoyl chloride were dissolved in 200 cc. toluene and refluxed until the evolution of hydrochloric acid ceased. The solution was cooled and washed with dilute sodium hydroxide and water. After drying with anhydrous calcium chloride the toluene was removed under reduced pressure and the residue crystallized from methyl alcohol, m. p. 88.5° C. (corr.).

3. ACTION OF ACID CHLORIDE ON THE PHENOL IN BENZENE WITH POTASSIUM CARBONATE.

5,7-Dichloro-8-Hydroxy-Quinoline Benzoate.—25 Gm. 5,7-dichloro-8-hydroxyquinoline, 17 Gm. benzoyl chloride and 50 Gm. anhydrous potassium carbonate (100 mesh) were dissolved in 250 cc. benzene. The mixture was refluxed and stirred for five hours. It was then treated for one-half hour with decolorizing carbon and filtered from excess potassium carbonate and potassium chloride. The precipitate was washed with hot benzene and the filtrate and washings combined. They were then washed with 5 per cent sodium hydroxide and with water and dried with calcium chloride. The solvent was distilled off and the residue recrystallized from alcohol, m. p. 129.5–130.5° C. (corr.).

4. REACTION BETWEEN THE ACID CHLORIDE AND THE PHENOL IN PYRIDINE.

2-Phenyl-Phenyl Acetyl-Salicylate.—14.7 Gm. 2-phenyl-phenyl salicylate were dissolved in 12 Gm. dry pyridine by the aid of heat and the solution cooled in an ice bath. To the resulting suspension 5 Gm. acetyl chloride were added dropwise and the reaction completed by warming on the water-bath for thirty minutes. The mixture was then poured upon ice and the resulting sticky product washed with water. It was recrystallized from alcohol until constant m. p. was reached. Consistently low yields of about 40% were obtained.

5. ACID, PHENOL AND PHOSPHORUS TRICHLORIDE OR PHOSPHORUS OXYCHLORIDE IN TOLUENE.

2-Phenyl-Phenyl Benzoate.—0.2 mol 2-phenyl-phenol and 0.2 mol benzoic acid were dissolved in 400 cc. toluene and heated under reflux. The solution was stirred mechanically to promote even boiling. 0.1 mol phosphorus oxychloride was added slowly and the refluxing and stirring continued until evolution of hydrochloric acid ceased. (This varied from three to eight

hours in various cases.) The solution was then cooled, decanted from the phosphoric acid, and washed with dilute sodium hydroxide and with water. After drying with calcium chloride the toluene was removed under reduced pressure and the residue crystallized from methyl alcohol as prisms, m. p. 75° C.

Preparation of Potassium 4-n-Propyl-2-Phenyl-Phenyl Sulphate.—16 Gm. pyridine were dissolved in 100 cc. carbon bisulphide and the solution cooled in an ice bath. 12 Gm. chlorosulphonic acid were added with good stirring during ten minutes. There was formed a crystalline precipitate of the double compound of pyridine and chlorosulphonic acid. 21 Gm. of 4-*n*-propyl-2-phenyl-phenol in an equal volume of carbon bisulphide were then added all at once. After heating for one hour on the steam-bath the carbon bisulphide was distilled off and 10 per cent potassium hydroxide added until strongly alkaline to litmus. The solution was then concentrated and allowed to crystallize. The crystals were washed with ice-cold water and then with alcohol and dried *in vacuo*. The yellow crystalline powder was readily soluble in water and slightly soluble in cold alcohol. It decomposed without melting at 180–190° C.

Preparation of Potassium 6-Chloro-2-Phenyl-Phenyl Sulphate.—This salt was prepared by the method described for the corresponding propyl compound. It formed a yellowish white crystalline powder which decomposed on heating.

Both of these sulphuric esters in water gave practically neutral solutions which slowly developed alkalinity upon standing.

Ester.	Crystallized from.	M. P., ° C. (Corrected).	Analysis Sought.	Found.	Calculated.
2-Phenyl-phenol					
Benzoate	Methyl alcohol	75–76	C	82.8	83.2
			H	5.10	5.11
Salicylate	Alcohol	91–92	C	78.1	78.6
			H	4.80	4.83
Cinnamate	Alcohol	103–104	C	83.7	84.0
			H	5.29	5.33
β-Resorcylate	Alcohol	185–186	C	74.6	74.7
			H	4.8	4.8
Acetyl Salicylate	Methyl alcohol	71.5–72.5	C	75.0	75.9
			H	4.82	4.83
3-Phenyl-phenol					
Benzoate	Alcohol	57–58	C	81.4	83.2
			H	5.11	5.11
4-Phenyl-phenol					
Benzoate	Benzene	148.5–149.5	C	83.0	83.2
			H	5.16	5.11
6-Chloro-2-phenyl-phenol					
Benzoate	Alcohol	86–87	Cl	11.68	11.51
Salicylate	Methyl alcohol	107	Cl	10.77	10.94
Cinnamate	Methyl alcohol	74.5–75	Cl	10.52	10.61
4-Chloro-2-phenyl-phenol					
Benzoate	Methyl alcohol	88.5	Cl	11.14	11.51
4-Bromo-2-phenyl-phenol					
Benzoate	Uncrystallizable oil	....	Br	22.77	22.64
x-Chloro-3-phenyl-phenol					
Benzoate	Uncrystallized thick oil	....	Cl	11.38	11.51
2-Chloro-4-phenyl-phenol					
Benzoate	Alcohol	110–111	Cl	11.26	11.51

4-n-Propyl-2-phenyl-phenol					
Benzoate	Petroleum ether	96	C	84.2	83.5
			H	6.35	6.33
Cinnamate	Petroleum ether	72.5	C	84.7	84.2
			H	6.39	6.44
5,7-Dichloro-8-hydroxyquinoline					
Benzoate	Alcohol	129.5–130.5	Cl	22.09	22.33
K salt of 4-n-propyl-2-phenyl-phenyl hydrogen sulphate	Water	Decomposes 180–190	S	9.07	9.69
K salt of 6-chloro-2-phenyl-phenyl hydrogen sulphate	Water	Decomposes 225–230	S Cl	9.80 10.29	9.92 10.98

The biological tests on compounds reported herein were made in the Biological Research Laboratories of E. R. Squibb and Sons and we gratefully acknowledge their assistance.

SUMMARY.

A number of esters of the three phenols and some of their substitution products were prepared and shown to have no value as urinary antiseptics.

REFERENCES.

(1) Leonard, V., *J. A. M. A.*, 83, 2005 (1925).
(2) Baumann, *Ber.*, 9, 55 (1876).

SALIVA TESTS. III. DETECTING THE ADMINISTRATION OF SOME OPIUM DERIVATIVES TO HORSES.*

BY JAMES C. MUNCH.[1]

Previous papers in this series (1, 2) report the development of a method for detecting morphine and heroin in the saliva of horses after the subcutaneous or intramuscular injection of known drugs (that is, the investigators knew that the horses had received morphine or heroin at the time tests upon mice were conducted).

TABLE I.—THRESHOLDS FOR MOUSE TESTS WITH OPIUM ALKALOIDS.

Product.	Mg./Kg.	10. Med. 70%. Effective Dose. Gamma/20-Gm. Mouse.
Morphine	4.00	80
Codeine	3.00	60
Dionine	1.00	20
Dilaudid	0.6	12
Heroin	0.05	1

The normal salivas collected from over one hundred untreated horses have been injected into mice; in no instance has an effect been observed resembling that produced by the opium alkaloids. The solution obtained by dissolving morphine or heroin in such a saliva, or in normal horse serum, produced the same effects

* Scientific Section A. PH. A., Washington meeting, 1935.

[1] Sharp and Dohme, Philadelphia, Penna.