CCXXV. SOME NEW ANTISEPTICS RELATED TO INDAN-1:3-DIONE.

BY FRANK ARNOLD ROBINSON, ARTHUR JAMES SUTHERS
AND THOMAS KENNEDY WALKER.

From the College of Technology, University of Manchester.

(Received September 29th, 1932.)

MANY compounds containing the 1:3-diketo-system, or the enolic form of the same, have been found to possess definite forms of physiological activity, and such properties may be particularly evident in molecules in which the 1:3-diketo-grouping constitutes part of a ring structure to which one or more alkyl or acyl groups are attached in certain positions. Cases in point are furnished by the hypnotic derivatives of barbituric acid, in which a *gem*-dialkyl group is situated between the two carbonyls; further instances are those of filicic acid and aspidinol, nuclear units of complex and more active substances present among the constituents of *Aspidium filix-mas* and of other ferns [*cf.* Henry, 1923].

The nucleus of filicic acid contains the grouping

and possesses a substituent acyl group attached to carbon atom number 4. Recognition of the presence of such a system in the naturally occurring anthelmintics has led to the examination of the properties of synthetic acyl derivatives of resorcinol and of phloroglucinol [Karrer and Rosenfeld, 1921] and these have been found to possess different degrees of anthelmintic activity.

Alkyl derivatives of resorcinol and of phloroglucinol, particularly a hexyl derivative of the former [Johnson and Lane, 1921; Leonard, 1924; Dohme, Cox and Miller, 1926; Klarmann, 1926; Klarmann and Figdor, 1926] possess marked antiseptic potency and it is noteworthy that lupulon, a highly antiseptic phloroglucinol derivative occurring in the soft resin of hops [Walker, 1924; 1925; Wöllmer, 1925; Wieland, Marz and Hoek, 1925; Wieland and Marz, 1926; Pyman, 1927], bears a very close structural relationship to filicic acid, an additional indication that antiseptic activity of a certain kind and anthelmintic activity are properties connected with the particular type of molecular architecture possessed by these substances.

Consideration of these and of similar relationships led one of us to make further investigation of the extent to which the 1:3-diketo-system, in conjunction with other groups, can condition or enhance physiological activity,

particularly antiseptic potency, and recently it was demonstrated [Black, Shaw and Walker, 1931; Walker *et al.*, 1931] that a number of derivatives of indan-1 : 3-dione, produced by attachment of the malonyl or mono-*n*-alkylmalonyl group to the nuclei of phenols, exert considerable bacteriostatic effects upon certain micro-organisms. In three cases it was shown that, starting from monoethylmalonyl chloride, the coupling of an homologous series of mono-*n*-alkylmalonyl chlorides with a given phenol resulted in the production of an homologous series of 2-*n*-alkylindan-1 : 3-diones of progressively increasing antiseptic power; and in the series derived from *p*-cresol such increase in power attained its limit with the compound containing the *n*-heptyl radical.

In view of this behaviour and of the fact that similar progressive increase of antiseptic potency has been noted in the homologous series of *n*-alkylresorcinols, where it culminates in the *n*-hexyl compound [Dohme, Cox and Miller, 1926; Klarmann, 1926], it appeared probable that condensations of *n*-amyl- and *n*-hexylmalonyl chlorides with suitable *n*-amyl, *n*-hexyl and *n*-heptyl derivatives of phenol would give rise to indan-1 : 3-diones of considerably greater antiseptic potency than those hitherto prepared.

Accordingly, a series of *p*-*n*-alkylphenols was synthesised and coupled with various *n*-alkylmalonyl chlorides, and investigation of the properties of the products has fully justified expectations as to their degrees of antiseptic activity. It was also intended originally to apply this reaction for the production of indan-1 : 3-diones to *C*-alkyl derivatives of *o*-, *m*-, and *p*-cresol but, whilst experiments with 3-*n*-amyl-*p*-cresol and indan-1 : 3-diones derived from it were in progress, Coulthard, Marshall and Pyman [1930] published an account of the syntheses and germicidal activities of several homologous series of phenols, including *C*-alkyl derivatives of *o*-, *m*- and *p*-cresol, and the data given in their paper have rendered unnecessary further work on the lines we had in view.

The constitutions of the new indan-1 : 3-diones and the numbers assigned to them for reference purposes are shown in Tables II and III, compounds I to XIV, inclusive, possessing structure (A), and compounds XV to XVIII, inclusive, possessing structure (B). Compound (I) was prepared during the course of a previous investigation [Walker *et al.*, 1931] and is included here in order to complete series I to VII.

The new substances exercise selective antiseptic action, for whilst they inhibit, even in very low concentrations, the proliferation of many Gram-positive bacilli and cocci, they exert little or no effect on the reproduction of certain Gram-negative organisms, relatively large doses of the substances failing to restrain the development of *Bact. coli commune*, *Bact. pyocyaneum* and *Bact. prodigiosum*, in broth or other media.

Table I permits comparison of the bacteriostatic powers of the members of the series of *p*-*n*-alkylphenols and of that of 3-*n*-amyl-*p*-cresol, whilst comparisons of the powers of the new indan-1 : 3-diones prepared from these substances may be made from the data in Tables II and III. The inhibiting

Table I.

Substance	Bacteriostatic concentration expressed as 1 g. of substance in x cc. of medium	Equimolecular phenol coefficient of bacteriostatic power	Equimolecular phenol coefficient of bactericidal power (calculated from phenol coefficients obtained by use of Rideal-Walker method)
Phenol	1 in 660	—	—
<i>p</i> -Cresol	1 in 1,580	2.7	2.87
<i>p</i> -Ethylphenol	1 in 1,660	3.2	9.7
<i>p</i> - <i>n</i> -Propylphenol	1 in 8,300	16.2	28.9
<i>p</i> - <i>n</i> -Butylphenol	1 in 25,000	59.8	111.7
<i>p</i> - <i>n</i> -Amylphenol	1 in 76,900	189	181.4
<i>p</i> - <i>n</i> -Hexylphenol	1 in 125,000	355	170.4
<i>p</i> - <i>n</i> -Octylphenol	1 in 550,000	1827	—
3- <i>n</i> -Amyl- <i>p</i> -cresol	1 in 71,400	205	473

[The concentrations of *p*-*n*-amylphenol and *p*-*n*-hexylphenol necessary to suppress the proliferation of *Bact. coli commune* in broth (p_H 6.1) for 48 hours at 37° were found to be 1 in 8300 and 1 in 12,500, respectively, whilst under the same conditions and in the same space of time either compound at a concentration of 1 in 5000 was found to kill this organism. A concentration of 1 in 100,000 of *p*-*n*-octylphenol killed *Bacterium C* in 48 hours but *Bact. coli commune* was not killed in 48 hours by this compound at a concentration of 1 in 25,000 (saturated solution).]

Table II.

(A)		Bacteriostatic concentration, expressed as 1 g. of substance in x cc. of medium	Equimolecular phenol coefficient of bacteriostatic power
(I) $R = CH_3$	$R' = n-C_4H_9$	1 in 45,000 { 1 in 9,080 at p_H 6.7 } 1 in 125,000 { 1 in 3,630 at p_H 7.7 }	170
(II) $R = C_2H_5$		1 in 222,000	490
(III) $R = n-C_3H_7$		1 in 555,000	920
(IV) $R = n-C_4H_9$		1 in 1,250,000	2,430
(V) $R = n-C_5H_{11}$		1 in 3,333,000	5,740
(VI) $R = n-C_6H_{13}$		1 in 5,000,000	16,060
(VII) $R = n-C_8H_{17}$			26,330
(VIII) $R = n-C_4H_9$	$R' = C_2H_5$	1 in 50,000 { 1 in 20,000 at p_H 6.7 } 1 in 500,000 { 1 in 6,660 at p_H 7.7 }	196
(IX) $R = n-C_6H_{13}$		1 in 3,333,000	2,187
			16,060
(X) $R = n-C_5H_{11}$	$R' = n-C_5H_{11}$	1 in 5,000,000 { 1 in 2,500,000 at p_H 6.7 } 1 in 250,000 { 1 in 500,000 at p_H 7.7 }	25,212
(XI) $R = n-C_6H_{13}$		1 in 1,000,000 { 1 in 125,000 at p_H 6.7 } 1 in 5,000,000 { 1 in 62,500 at p_H 7.7 }	1,093
(XII) $R = C_2H_5$	$R' = n-C_6H_{13}$	1 in 1,250,000 { 1 in 1,250,000 at p_H 6.7 } 1 in 833,300 { 1 in 833,300 at p_H 7.7 }	5,044
(XIII) $R = n-C_5H_{11}$			26,330
(XIV) $R = n-C_6H_{13}$			

Table III.

(B)		Bacteriostatic concentration, expressed as 1 g. of substance in x cc. of medium	Equimolecular phenol coefficient of bacteriostatic power
(XV) $R' = n-C_5H_9$		1 in 833,300	3,830
(XVI) $R' = n-C_6H_9$		1 in 1,666,600	8,030
(XVII) $R' = n-C_5H_{11}$		1 in 3,333,000	16,800
(XVIII) $R' = n-C_6H_{13}$		1 in 250,000	1,330

concentrations were determined by the turbidity method in lemco-peptone broth (p_H 6.1, unless otherwise stated), the organism employed being *Bacterium C* (National Collection of Type Cultures, Lister Institute). Incubation in the presence of different doses of each antiseptic was allowed to proceed for 48 hours at 37° and the results of all the tests were confirmed by triplicate determinations. *Bacterium C* was selected originally for the purpose after preliminary trials had shown that its behaviour towards this class of antiseptics is representative of that of a number of Gram-positive bacilli and cocci; moreover *Bacterium C* develops rapidly, it may be cultured over considerable periods of time without change of properties, and, in other ways, it is very suitable for use in determinations of bacteriostatic power.

In Tables I, II and III, the third column in each case shows the bacteriostatic powers of the several compounds compared with that of phenol and expressed on an equimolecular basis¹, *i.e.* equimolecular phenol coefficient of bacteriostatic power = (phenol coefficient of bacteriostatic power $\times$ molecular weight of substance) $\div$ molecular weight of phenol. The figures in the fourth column of Table I have been calculated from phenol coefficients given by Coulthard, Marshall and Pyman [1930], and they are shown here for purposes of comparison. It is of interest to note in the case of the *p-n*-alkylphenols that while both the bacteriostatic activity towards *Bacterium C* and the bactericidal activity towards *Bact. typhosum* increase with the number of carbon atoms in the side-chain, the bactericidal activity attains its maximum in the *p-n*-amyl derivative whereas the bacteriostatic activity continues to increase along the series beyond the *p-n*-amyl derivative and may not have attained its maximum in the *p-n*-octyl derivative, the highest member examined. The *p-n*-heptyl derivative of phenol was not prepared since oenanthic acid was not available at the time this work was undertaken.

Reference has already been made to the fact that in the homologous series of 4-hydroxy-7-methyl-2-*n*-alkylindan-1 : 3-diones antiseptic activity was found to increase progressively until it attained a maximum intensity in the 2-*n*-heptyl derivative. Similar behaviour is to be noted in two homologous series of this type shown in Tables II and III, respectively. In Table II, compounds IX, VI, XI and XIV are members of such an homologous series and it would appear that the antiseptic activity has culminated in XIV, where $R' = n$ -hexyl, since the equimolecular phenol coefficient of this compound is only very slightly greater than that of XI. The four compounds in Table III show very definitely the same kind of behaviour; here the 2-*n*-amyl derivative possesses the greatest antiseptic power, the 2-*n*-hexyl compound proving to be relatively weak.

Scrutiny of Table II also shows that progressive increase of antiseptic potency occurs in the series of compounds prepared by coupling a given alkylmalonyl chloride with an homologous series of *p-n*-alkylphenols, and if

¹ Morgan, Cooper and Burt [1923] have pointed out that it is preferable to compare phenol coefficients on an equimolecular basis.

we consider the series I to VII, inclusive, it is seen that bacteriostatic potency is very considerably higher in the case of the 7-*n*-octyl derivative VII than in that of the 7-*n*-hexyl derivative VI. Since the 7-*n*-heptyl and the 7-*n*-nonyl derivatives were not prepared, it is not possible to decide from the present data whether the 7-*n*-octyl derivative is the most potent compound of this type which could be synthesised. Further synthetical work in this series was not practicable, however, for great difficulty was encountered in separating pure substances from the highly resinous reaction products which were obtained when components containing these larger alkyl groups were employed in the condensation.

*Bacteriostatic and bactericidal action of indan-1:3-diones
on a variety of micro-organisms.*

In order to obtain information on broader lines concerning the inhibitory and lethal effects of the new indan-1:3-diones, three of these compounds (*viz.* X, XIV and XVII) which, in tests against *Bacterium C*, had exhibited considerable bacteriostatic powers, were employed in experiments with the following organisms: *Bact. pyocyaneum* Flügge (Fildes III), *Bact. prodigiosum* Cohn, *Bact. coli* Escherich, *Staphylococcus albus* Rosenbach, *Bac. megatherium* de Bary, *Bac. subtilis* (Ehrenberg) Cohn, *Bac. mycoides* Flügge and two strains of *Mycobact. phlei* (Möller) Lehmann and Neumann. These organisms, with the exception of the specimen of *S. albus*, were obtained from the National Collection of Type Cultures, Lister Institute.

The determinations of bacteriostatic and bactericidal activity were made by the turbidity method previously employed in the case of *Bacterium C*, living organisms being sought for in subcultures when visual inspection of the tubes failed to detect growth. The results are shown in Table IV where, for purposes of comparison, the effects of these substances on *Bacterium C* are also reproduced.

These determinations have served to emphasise the highly selective action of the substances, since *Bact. pyocyaneum*, *Bact. prodigiosum* and *Bact. coli* were not prevented from multiplying by concentrations many times greater than those found necessary to kill the organisms cited in Table IV. Marked selectivity is also seen in the cases of *S. albus* and *M. phlei*, compound X exerting intense bacteriostatic effects upon the former organism, while the latter organism appears to be particularly susceptible to the influence of compound XIV.

It was not considered necessary to determine the exact bactericidal concentration in more than half of the cases studied; in other instances it was ascertained whether the lethal dose lay above or below a given figure. The data shown in Table IV are adequate, however, to indicate that in some cases (*e.g.* that of *M. phlei*) the dose of antiseptic which is just sufficient to inhibit growth over a period of 48 hours is also lethal in that time, that is to say, in such cases the germicidal concentration is only slightly higher than the highest

Table IV. Results illustrating the bacteriostatic and bactericidal activities of indan-1:3-dione derivatives towards various micro-organisms.

(a) = bacteriostatic concentration in broth (p_H 6.1) at 37° for 48 hours; (b) = bactericidal concentration in broth (p_H 6.1) at 37° for 24 hours.

Organism	<div style="text-align: center;"> (X) </div>		<div style="text-align: center;"> (XIV) </div>		<div style="text-align: center;"> (XVII) </div>	
	(a)	(b)	(a)	(b)	(a)	(b)
* <i>Bacterium C</i>	1: 3,333,000	>1: 166,000	1: 5,000,000	<1: 166,000	1: 3,333,000	<1: 166,000
<i>Bac. megatherium</i>	1: 2,500,000	>1: 500,000	—	—	1: 2,500,000	>1: 1,666,000
<i>Bac. subtilis</i>	1: 2,500,000	<1: 500,000	1: 5,000,000 (1: 1,250,000 at p_H 7.7)	1: 1,430,000 (1: 833,000 at p_H 7.7)	1: 2,500,000	>1: 1,000,000
<i>Bac. mycoides</i>	1: 2,500,000	<1: 500,000	1: 5,000,000 (1: 1,250,000 at p_H 7.7)	1: 2,000,000 (1: 1,250,000 at p_H 7.7)	1: 3,333,000	1: 1,666,000
<i>S. albus</i>	<1: 10,000,000	<1: 500,000	1: 3,333,000 (1: 1,000,000 at p_H 7.7)	1: 1,666,000 (1: 833,000 at p_H 7.7)	1: 2,500,000	1: 1,666,000
<i>M. phlei</i>	(i)	(i)	(i)	(i)	(i)	(i)
(i) strain 525	1: 5,000,000	<1: 500,000	1: 11,110,000	1: 11,110,000	1: 3,333,000	1: 3,333,000
(ii) strain 54			(ii) 1: 20,000,000	(ii) 1: 20,000,000	(ii) 1: 3,333,000	(ii) 1: 2,000,000
			(ii) (1: 1,428,000 at p_H 7.7)	(ii) (1: 1,428,000 at p_H > 7.7)		

* This unclassified organism was designated provisionally as *Bacterium C* by Chapman but, since it gives rise to spores it could be designated, according to the convention of Lehmann and Neumann, as *Bacillus C*.

concentration of antiseptic in the presence of which growth can occur with comparative freedom. In other cases, according to the particular organism, the concentration of antiseptic necessary to produce germicidal effects within 24 hours may be as much as 20 times higher than that which is sufficient to inhibit growth during the same period (for example, *Bacterium C* and compound X).

In view of these results it was thought of interest to obtain further information as to the intensity of the bactericidal activity of the substances towards Gram-positive organisms and, accordingly, the action of compound XI on *Bac. megatherium*, *Bac. subtilis*, *Bac. mycoides* and *S. albus* was studied under the conditions of the Rideal-Walker method. The substance (0.1 g.) was first dissolved in 100 cc. of 0.02 *N* Na₂CO₃ and, by dilution of aliquot portions of this stock solution, with sterile water, the concentrations required were obtained (Table V).

The bactericidal action of these indan-1:3-diones is seen, therefore, to be rapid at higher concentrations, and still reasonably rapid even at low concentrations, and, generally, in their antiseptic effects the substances are strikingly similar to the triphenylmethane dyes of the type of crystal violet which, as Churchman [1926] has shown, are much more powerfully bacteriostatic towards Gram-positive organisms such as staphylococcus, diphtheroids and

Table V.

Organism	Time, in minutes, necessary for completion of the lethal action of compound XI at concentrations of		
	1 : 10,000	1 : 100,000	1 : 1,000,000
<i>Bac. megatherium</i>	<10	—	<30
<i>Bac. subtilis</i>	<10	<30	—
<i>Bac. mycoides</i>	<10	<60	—
<i>S. albus</i>	<10	—	<60
<i>Bact. coli</i> was killed in 30 minutes but not in 15 minutes by compound XI at a concentration of 1 : 1000.			

aerobic spore-forming bacilli than towards Gram-negative organisms like *Bact. coli*. With the indan-1 : 3-diones, however, this selectivity of action is decidedly more pronounced than with the triphenylmethane dyes, and this fact would appear to indicate that the former compounds could be utilised very successfully in the separation of coliform organisms from certain mixed cultures.

*The effect of hydrogen ion concentration on the antiseptic activity of
indan-1 : 3-diones.*

Tables II and IV contain sufficient data concerning the effects of hydrogen ion concentration to indicate clearly that the antiseptic activities of the substances examined are greatly diminished by adjustment of the medium from the acid range of the scale towards the neutral point, and that this effect becomes still more marked when the medium is rendered faintly alkaline. It is also evident that no general statement can be made regarding the relative effects likely to be produced by alterations in the reaction of the medium, since two compounds which exercise approximately equal inhibitory actions upon a given organism at one particular hydrogen ion concentration, may manifest widely divergent activities towards the same organism at other hydrogen ion concentrations.

The effect of serum on the antiseptic activity of indan-1 : 3-diones.

It was considered desirable to ascertain the effect of serum upon the bactericidal activities of indan-1 : 3-dione derivatives, and Prof. C. H. Browning very kindly undertook to examine one of the new compounds from this point of view. The substance chosen was 4-hydroxy-7-n-hexyl-2-n-amyli-*indan-1 : 3-dione* (compound XI) which, as previous tests had shown, is one of the most potent of the new compounds. He reported as follows:

“0.05 g. was dissolved in 5 cc. alcohol and 5 cc. water added; the precipitate which formed was dissolved by the addition of a drop of Na_2CO_3 solution. This 0.5 % stock solution was stable and from it further dilutions in water were made. A volume not exceeding 0.1 cc. of various dilutions was then added to a series of tubes each containing 1 cc. 0.7 % peptone water medium or ox-serum (previously heated at 56°), so as to yield a wide range of concentrations of the antiseptic. In peptone water precipitation occurred

with 1 : 20,000 of the substance, but 1 : 2000 in serum gave no precipitate. The tubes were then inoculated with 0.1 cc. of a 1 : 1000 dilution in saline of a 24 hours' fluid culture of *S. aureus* or *Bact. coli*. The results were read after 48 hours' incubation at 37°, both naked-eye evidence of growth and the presence of living organisms, as tested by subculture, being determined.

The *Staphylococcus* in peptone water was sterilised by 1 : 400,000, but in serum 1 : 2000 failed to sterilise. In the case of *Bact. coli*, 1 : 2000 failed to sterilise in peptone water and in serum."

Syntheses of acylphenols and reduction of these to alkylphenols.

The *p-n*-alkyl derivatives of phenol examined by Coulthard, Marshall and Pyman [1930] were obtained by reduction of the corresponding *p*-acylphenols by Clemmensen's method. These authors prepared *p*-propionyl, *p-n*-valeryl and *p-n*-hexoylphenols by heating phenol with the appropriate fatty acid and anhydrous zinc chloride, a process due originally to Nencki. We found that much better yields of these three ketones were obtained by employing the Fries isomerisation, a method used by Coulthard, Marshall and Pyman [1930] to obtain the corresponding derivatives of the three cresols. In our experiments the acid chloride (1 mol.) was added to phenol (1 mol.) and 30 minutes later the mixture was warmed on a water-bath for 60 minutes. The resulting ester of the phenol was then diluted with three times its volume of freshly distilled nitrobenzene, and aluminium chloride (1.1 mols.) in a finely powdered condition was added in small quantities at a time. The reaction was completed by heating on a water-bath for 60 minutes. The material was then submitted to steam-distillation in order to remove nitrobenzene and to separate the volatile *o*-hydroxyarylketone from the non-volatile *p*-isomeride. The latter was isolated by extraction with ether and distillation under low pressure. The yields were: *p*-propionylphenol, 25 %; *p-n*-butyrylphenol, 18 % (Coulthard, Marshall and Pyman obtained 19 % in absence of nitrobenzene); *p-n*-valerylphenol, 37 %; *p-n*-hexoylphenol, 58 %.

The preparation of 3-*n*-valeryl-*p*-cresol (65 % yield) was carried out under the conditions used by Coulthard, Marshall and Pyman, *i.e.* the isomerisation was conducted at 160° in absence of nitrobenzene.

p-Acetylphenol was prepared according to a method described by Nencki [1897], which consisted in treating a mixture of phenol and acetyl chloride in carbon disulphide solution with ferric chloride. The yield was 60 %. The reduction of the above-mentioned *p*-hydroxyarylketones to the corresponding alkylphenols by Clemmensen's method was effected in every case with a yield of 80 % or over.

p-n-Octylphenol has been described recently by Sandulesco and Girard [1930]. We prepared it as follows. *n*-Caprylic acid (73 g.) on treatment with thionyl chloride (88 g.) gave 57 g. (70 % yield) of the acid chloride, which boiled at 101–102°/18 mm. This was added to freshly distilled phenol (33 g.) and, after the resulting reaction had moderated, the mixture was warmed for

60 minutes on a steam-bath and subsequently diluted with 200 cc. of freshly distilled nitrobenzene. Finely powdered anhydrous aluminium chloride (52 g.) was now added in small portions, and the reaction was completed by heating for an hour at 75°. The reaction product was poured on ice, treated with hydrochloric acid and submitted to steam-distillation. The non-volatile organic residue was taken up in ether and was subsequently fractionated under reduced pressure. Some material which distilled up to 180° at 13 mm. probably contained a little *o-n*-caprylylphenol; the *p-n*-caprylylphenol subsequently passed over at 215 to 220°/15 mm. and solidified on standing. This crude fraction (30 g., *i.e.* 40 % yield) was not further purified but was reduced by heating with 100 g. of amalgamated zinc-wool and 600 cc. of dilute hydrochloric acid (1 vol. concentrated acid : 2 vols. water) on a water-bath at 100° during 16 hours, the acid being added in portions from time to time. (The zinc-wool had been amalgamated by treatment for 5 minutes with a 5 % aqueous solution of mercuric chloride.) The reduction product, isolated by extraction with ether, was distilled under reduced pressure and the fraction boiling at 195–205°/15 mm. (Sandulesco and Girard give 169°/10 mm.) was collected. This crystallised in colourless needles, on standing, and the yield was 55 %. Owing to the fact that the melting-point was low and indefinite (*ca.* 41°) the substance was characterised as the *p*-nitrobenzoate, which was prepared by heating 3 g. of the phenol with 3 g. of *p*-nitrobenzoyl chloride in 20 cc. of pyridine on a water-bath for 2 hours. The reaction mixture was poured into dilute hydrochloric acid and the solid product was separated, dried and crystallised from acetone. It melted at 81.5°. (Found, by micro-method: N, 4.07 %, $C_{21}H_{25}O_4N$ requires N, 3.94 %.)

A communication by Sandulesco and Girard [1930], which was published almost simultaneously with that of Coulthard, Marshall and Pyman, also contains an account of the syntheses of *o*- and *p*-acyl derivatives of phenol and of their subsequent reduction to the corresponding alkyl derivatives, the methods employed being very similar to those used independently by us and by Coulthard, Marshall and Pyman. Sandulesco and Girard state that somewhat higher yields of the *p*-acyl derivatives of phenol are obtained when working in the absence of nitrobenzene. Their yields of *p*-alkyl derivatives of phenol obtained by use of Clemmensen's reduction method are of the same order as those obtained by us.

Syntheses of 4-hydroxyindan-1:3-diones.

A general method, and modifications of the same, for the preparation of substances of this class were described in a former communication [Walker *et al.*, 1931]. The 17 new compounds examined in the course of the present investigation were all prepared by this same general process, namely, condensation of an alkylmalonyl chloride with an alkylphenol in nitrobenzene solution in the presence of anhydrous aluminium chloride. In some cases, however, difficulties were encountered in purifying the product, and this was

the case notably in those instances where *R* and *R'* were alkyl groups containing more than four carbon atoms. Increase in length of the alkyl chain in the malonyl chloride component was accompanied by an increasing tendency to resin formation, particularly if the reaction in nitrobenzene solution was conducted above 80° and, on the other hand, the employment of a temperature lower than 70° resulted in a low yield since a smaller percentage of the components interacted. In this latter event the product was contaminated with relatively large amounts of alkylmalonic acid and unchanged alkylphenol, and the latter, owing to low volatility, could not be removed in steam. In such cases the following procedure was adopted for the isolation and purification of the indan-1 : 3-dione. The nitrobenzene solution of the reaction products was poured on to ice and treated with sufficient hydrochloric acid to take the aluminium into solution. The nitrobenzene was removed in steam and the cooled residue was extracted thoroughly with ether. The ethereal extract was then washed many times with water to remove all traces of alkylmalonic acid, after which it was extracted very thoroughly with successive portions of a large bulk of a 7.5 % aqueous solution of sodium carbonate, followed by an extraction with cold water. This removed the indan-1 : 3-dione in the form of its sodium salt, and unchanged alkylphenol was left in the ether. The combined sodium carbonate extracts together with the final aqueous extract were acidified with hydrochloric acid and the free indan-1 : 3-dione was taken up in ether when, after drying and removal of the ether, it solidified and was recrystallised from appropriate solvents.

All the new indan-1 : 3-diones are colourless solids which are practically insoluble in cold water and soluble in aqueous solutions of alkali metal hydroxides or alkali metal carbonates. They are generally soluble in, and crystallise well from, the usual organic solvents, the most suitable of which for purposes of purification were found to be acetone and methyl alcohol in the hot, and an acetone-light petroleum mixture.

The analytical data and melting-points are given in Table VI. The combustions were performed by the micro-method.

Table VI.

Substance (see Tables II and III)	Formula	M.P.	Found		Calculated	
			% C	% H	% C	% H
4-Hydroxy-7-ethyl-2- <i>n</i> -butylindan-1:3-dione	(II) C ₁₅ H ₁₈ O ₃	111°	73.0	7.5	73.2	7.3
4-Hydroxy-7- <i>n</i> -propyl-2- <i>n</i> -butylindan-1:3-dione	(III) C ₁₆ H ₂₀ O ₃	122-123	74.5	8.1	73.8	7.7
4-Hydroxy-2:7-di- <i>n</i> -butylindan-1:3-dione	(IV) C ₁₇ H ₂₂ O ₃	120	74.9	7.9	74.4	8.0
4-Hydroxy-7- <i>n</i> -amyl-2- <i>n</i> -butylindan-1:3-dione	(V) C ₁₈ H ₂₄ O ₃	110	75.2	8.4	75.0	8.3
4-Hydroxy-7- <i>n</i> -hexyl-2- <i>n</i> -butylindan-1:3-dione	(VI) C ₁₉ H ₂₆ O ₃	118-119	76.0	8.7	75.5	8.6
4-Hydroxy-7- <i>n</i> -octyl-2- <i>n</i> -butylindan-1:3-dione	(VII) C ₂₁ H ₃₀ O ₃	105	76.4	9.35	76.4	9.1
4-Hydroxy-7- <i>n</i> -butyl-2-ethylindan-1:3-dione	(VIII) C ₁₅ H ₁₈ O ₃	129	73.2	7.3	73.2	7.3
4-Hydroxy-7- <i>n</i> -hexyl-2-ethylindan-1:3-dione	(IX) C ₁₇ H ₂₂ O ₃	120	74.1	8.4	74.4	8.0
4-Hydroxy-2:7-di- <i>n</i> -amylindan-1:3-dione	(X) C ₁₉ H ₂₆ O ₃	114-115	75.2	9.0	75.5	8.6
4-Hydroxy-7- <i>n</i> -hexyl-2- <i>n</i> -amylindan-1:3-dione	(XI) C ₂₀ H ₂₈ O ₃	100	75.8	8.9	75.9	8.8
4-Hydroxy-7-ethyl-2- <i>n</i> -hexylindan-1:3-dione	(XII) C ₁₇ H ₂₂ O ₃	110	74.6	7.9	74.4	8.0
4-Hydroxy-7- <i>n</i> -amyl-2- <i>n</i> -hexylindan-1:3-dione	(XIII) C ₂₀ H ₂₈ O ₃	98-99	75.5	9.0	75.9	8.8
4-Hydroxy-2:7-di- <i>n</i> -hexylindan-1:3-dione	(XIV) C ₂₁ H ₃₀ O ₃	95-96	76.0	9.3	76.4	9.1
4-Hydroxy-5- <i>n</i> -amyl-7-methyl-2- <i>n</i> -propylindan-1:3-dione	(XV) C ₁₈ H ₂₄ O ₃	149	75.5	8.0	75.0	8.3
4-Hydroxy-5- <i>n</i> -amyl-7-methyl-2- <i>n</i> -butylindan-1:3-dione	(XVI) C ₁₉ H ₂₆ O ₃	119-120	76.1	8.9	75.5	8.6
4-Hydroxy-7-methyl-2:5-di- <i>n</i> -amylindan-1:3-dione	(XVII) C ₂₀ H ₂₈ O ₃	90-92	76.1	8.6	75.9	8.8
4-Hydroxy-5- <i>n</i> -amyl-7-methyl-2- <i>n</i> -hexylindan-1:3-dione	(XVIII) C ₂₁ H ₃₀ O ₃	143-144	76.3	9.1	76.4	9.1

SUMMARY.

1. Seventeen new alkyl derivatives of 4-hydroxyindan-1 : 3-dione, all of which possess antiseptic power, have been prepared by the condensation of mono-*n*-alkylmalonyl chlorides with *p*-*n*-alkyl derivatives of phenol and with 3-*n*-amyl-*p*-cresol, in the presence of aluminium chloride.

2. Examination of these products shows that when a given mono-*n*-alkylmalonyl chloride is condensed with the lower members of the homologous series of the *p*-*n*-alkyl derivatives of phenol, the antiseptic powers of the members of the resulting homologous series of 4-hydroxyindan-1 : 3-diones increase progressively as the series is ascended. The limits of such increases have not been determined in the present investigation.

3. Two sets of results now obtained confirm a former observation that the condensation of several consecutive lower members of the homologous series of mono-*n*-alkylmalonyl chlorides with an appropriate phenol results in the formation of an homologous series of hydroxyindan-1 : 3-diones, the members of which exhibit antiseptic power which increases progressively to a maximum value as the series is ascended. Such maximum value appears usually to be attained in the 2-*n*-amyl-, 2-*n*-hexyl-, or 2-*n*-heptyl-compound.

4. The antiseptic activity of these 4-hydroxyindan-1 : 3-diones is highly selective in character. In very low concentrations they inhibit the proliferation of Gram-positive bacilli and cocci, as shown by tests against *Bac. megatherium*, *Bac. subtilis*, *Bac. mycoides*, *S. albus* and *S. aureus*. At higher concentrations the substances exercise lethal action on all these organisms. The acid-fast organism *Mycobact. phlei* is particularly sensitive to the presence of certain of them; thus one strain, in broth (p_H 6.1), is killed within 24 hours at 37° when 4-hydroxy-2 : 7-di-*n*-hexylindan-1 : 3-dione is present at a concentration of 1 : 20,000,000. Another strain of the same organism is killed under the same conditions when the same compound is present at a concentration of 1 : 11,110,000.

On the other hand these compounds have little or no adverse action on the proliferation, in broth, of the Gram-negative organisms *Bact. coli*, *Bact. pyocyaneum* and *Bact. prodigiosum*.

5. The antiseptic activities of the new substances are depressed considerably by culture media which are slightly alkaline in reaction, and are suppressed entirely by ox-serum.

REFERENCES.

- Black, Shaw and Walker (1931). *J. Chem. Soc.* 272.
Churchman (1926). *Stain Technology*, **1**, 27.
Coulthard, Marshall and Pyman (1930). *J. Chem. Soc.* 280.
Dohme, Cox and Miller (1926). *J. Amer. Chem. Soc.* **48**, 1688.
Henry (1923). *Pharm. J.* 28.
Johnson and Lane (1921). *J. Amer. Chem. Soc.* **43**, 348.
Karrer and Rosenfeld (1921). *Helv. Chim. Acta*, **4**, 707.
Klarman (1926). *J. Amer. Chem. Soc.* **48**, 2358.
—— and Figdor (1926). *J. Amer. Chem. Soc.* **48**, 791.
Leonard (1924). *J. Amer. Med. Assoc.* **83**, 2005.
Morgan, Cooper and Burt (1923). *Biochem. J.* **17**, 30.
Nencki (1897). *Ber. deutsch. chem. Ges.* **30**, 1768.
Pyman (1927). *J. Inst. Brew.* **33**, 295.
Sandulesco and Girard (1930). *Bull. Soc. Chim.* (iv), **47**, 1300.
Walker (1924). *J. Inst. Brew.* **30**, 570, 712.
—— (1925). *J. Inst. Brew.* **31**, 562.
—— Suthers, Roe and Shaw (1931). *J. Chem. Soc.* 514.
Wieland and Marz (1926). *Ber. deutsch. chem. Ges.* **59**, 2352.
—— ——— and Hoek (1925). *Ber. deutsch. chem. Ges.* **58**, 102, 2012.
Wöllmer (1925). *Ber. deutsch. chem. Ges.* **58**, 672.