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ANTITUBERCULOUS EFFECTS OF CERTAIN SURFACE-ACTIVE POLYOXYETHYLENE ETHERS

BY

J. W. CORNFORTH, P. D'ARCY HART, G. A. NICHOLLS, R. J. W. REES, AND J. A. STOCK

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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We have previously reported (Cornforth, Hart, Rees, and Stock, 1951) that a commercial surface-active agent, the non-ionic "Triton A20" (Rohm and Haas Co., Philadelphia),* has a suppressive effect on experimental tuberculosis in the mouse, and that the effect is shared by a number of similarly constituted products prepared by ourselves. These observations introduced a new type of antituberculous agent. Furthermore, since the therapeutically active products failed to inhibit the multiplication of tubercle bacilli *in vitro*, it was suggested that they might in some way modify the host's response to a tuberculous infection (Rees, 1953). The antituberculous properties of Triton A20 have been confirmed by Solotorovsky and Gregory (1952), who also reported a synergistic action with dihydrostreptomycin in a mouse infection. In addition to suppressing acute tuberculosis in mice, these agents can produce appreciable regression and healing of an established infection in the guinea-pig (Rees, 1953). The present paper supplies necessary details of the earlier work and describes further developments. Some of the results were briefly communicated by Cornforth, Hart, Nicholls, and Rees (1953).

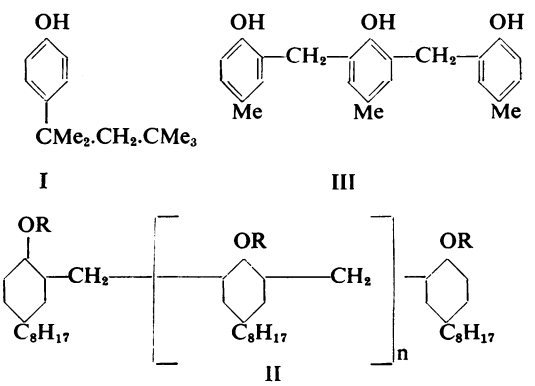
CHEMICAL SECTION

CHEMICAL NATURE OF PRODUCTS

Our first experiments, which were based on a chemical examination of Triton A20, and which resembled procedures given in U.S. Patent No. 2,454,541, involved acid-catalysed condensation of *p*-(1:1:3:3-tetramethylbutyl)phenol (I; referred to henceforward as octylphenol) with formaldehyde, followed by heat treatment of the product and reaction of the resulting resin with 17 to 20 molecules of ethylene oxide for each phenolic group. The final product, a water-soluble syrup, differed slightly in some of its properties from Triton A20, but proved equally effective against experimental tuberculosis in the mouse.

* The solute, of which Triton A20 is an aqueous solution, is known as WR1339

According to the patent cited above, a product thus prepared should have a general formula (II) and the octylphenol-formaldehyde condensation product should be (II; R = H). There is no reason to doubt



(R=$(CH_2CH_2O)_xH$, except where otherwise stated.)

the essential truth of this contention. The composition of our octylphenol-formaldehyde condensation product agreed with the expression (II; R = H). Though the product was insoluble in aqueous alkali, free phenolic groups could be demonstrated by the tautomeric shift in ultra-violet light absorption when alkali was added to an alcoholic solution. Finally, the acid-catalysed formation of linear polymers corresponding to formula (II; R = H) was shown, with *p*-cresol, by Megson and Drummond (1930), who isolated a tricyclic compound (III) from the reaction mixture.

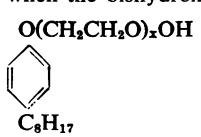
However, the condensation product of octylphenol with formaldehyde satisfies none of the ordinary criteria of purity. The antituberculous activity observed after condensation with ethylene oxide might, it was thought, be due to substances of different structure present in relatively small proportion. Even if the activity was indeed due to molecules of the general formula (II), it might still be associated with a particular value, or range of values, of *n*.

Under appropriate conditions, reaction of *p*-substituted phenols with formaldehyde can certainly lead

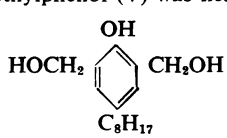
to products not expressible by formula (II ; R = H) ; for example, substances in which aromatic nuclei are joined by a dimethylene ether link, - CH_2 - O - CH_2 - , instead of the methylene link, - CH_2 -. Such substances are reported to be unstable to heat, and to afford benzyl bromides on treatment with hydrogen bromide in benzene (von Euler, Adler, Cedwall, and Törngren, 1942 ; Zinke, Tomio, and Lercher, 1942). Our products had been heated strongly during preparation, and they acquired no organically bound bromine on treatment with hydrogen bromide. Hence it is unlikely that ether linkages of this sort are present, and the same applies to hydroxymethyl groups, - CH_2OH. Yet the decomposition by heat of such *o*-hydroxybenzyl ethers or *o*-hydroxybenzyl alcohols can give rise to products among which chromans, diphenylethylenes, and diphenylethanes have been mentioned (Alder, von Euler, and Cedwall, 1942 ; Hultzsch, 1950). The possible presence of such substances in minor amount is more difficult to exclude except on the general ground that benzyl alcohols and benzyl ethers are unlikely to attain significant concentrations in an acid-catalysed condensation. These considerations made it very desirable to prepare, for condensation with ethylene oxide, pure substances of known structure.

Preparation of a series of substances having the structure (II ; R = H) in which n is 0, 1, 2 . . . , was an early objective : the parent of this homologous series is of course octylphenol (I) itself, which on condensation with ethylene oxide gave products of the general formula (IV). The dicyclic compound (n = 0), was easily obtained from formaldehyde and an excess of octylphenol. For the tricyclic compound (n = 1), 2:6-bishydroxymethyl-4-*tert.*-octylphenol (V) was required ; this has been made before (Sherman, 1938 ; U.S. Patent 2,488,134), but our own procedure is given in the experimental section. Two crystalline modifications of the substance were encountered.

Condensation of *p*-alkylphenols with substances of type (V) has been reported (Niederl and Ruderman, 1945) to give polymers higher than the tricyclic stage. This is true, and might have been expected, when equivalent quantities of the reactants are employed ; but when the bishydroxymethylphenol (V) was heated



IV



V

with an excess of octylphenol and a little trichloroacetic acid, the desired tricyclic substance (II ; R = H ; n = 1) was obtained without difficulty. It was isolated by distillation in high vacuum and was later obtained crystalline. Similar condensations of *p*-cresol with its bishydroxymethyl derivative have been described (Megson and Drummond, 1930 ; Koebner, 1933), hydrochloric acid being the catalyst. A trial of this catalyst gave a substantial amount of dicyclic material (II ; R = H ; n = 0), the strong acid evidently causing fission of a hydroxymethyl group.

Serious difficulties were met in attempts to make the pure tetracyclic compound (II ; R = H ; n = 2). A modification of Koebner's (1933) technique was used, excess of the dicyclic phenol (II ; R = H ; n = 0) being condensed with formaldehyde in acetic acid with hydrogen chloride. Excess of dicyclic phenol was removed by distillation ; short-path distillation in a molecular still then gave a preparation containing the desired product. This could not be crystallized. To obtain pure material, counter-current distribution was first tried ; the solvent pair was ligroin—94% ethanol. Though this operation provided evidence that the product of two successive molecular distillations was not homogeneous, material having a constant partition ratio was not obtained even after repeated distributions.

The pentacyclic compound (II ; R = H ; n = 3) was obtained by condensation of 2:6-bishydroxymethyl-4-*tert.*-octylphenol (V) with excess of dicyclic compound. The crude product was partially purified by molecular distillation.

Reversed-phase partition chromatography of the various condensation products described above was now examined, primarily as a method of testing the homogeneity of different preparations. Procedures were found for separation of the tetracyclic and pentacyclic phenols from each other and from lower homologues. It was shown that the tetracyclic compound was contaminated with lower homologues even after two molecular distillations and two counter-current distributions. The pentacyclic compound (undistilled) was similarly contaminated, and the tetracyclic compound was one of the impurities. Evidently this was formed by fission of a hydroxymethyl group, the resulting formaldehyde then coupling two molecules of dicyclic compound.

In principle, these separations on the milligram scale could have been applied to prepare larger amounts of pure materials for reaction with ethylene oxide ; but at this time we were attracted by another possibility. Zinke and Ziegler (1944) described a crystalline product, obtainable under special conditions from formaldehyde and *p-tert.*-butylphenol, to which a macrocyclic structure (VI ; R = CMe_3) was assigned. A later paper (Zinke, Zigeuner, Hössinger, and Hoffmann, 1948) reported, without characterization, similar products from certain other phenols. Repetition of the work on *p-tert.*-butylphenol demonstrated that two compounds could be separated from the crude crystalline product. Further, when the process was applied to octylphenol, two crystalline compounds were again isolated.

The two compounds from *p-tert.*-butylphenol were crystalline, high-melting, and sparingly soluble in most solvents. Analysis indicated for each an empirical formula $(C_{11}H_{14}O)_n$. It followed that the oxygen in the molecule all originated from *p-tert.*-butylphenol, and the preparation of crystalline acetates $(C_{11}H_{13}OAc)_n$ showed that all oxygen atoms were still

in the form of phenolic hydroxyl groups. The presence of a cyclic structure analogous to (VI), rather than a linear structure of type (II; R = H), was deducible from chemical evidence indicating the absence of a free position *ortho* to a hydroxyl group: such positions are present at each end of a linear polymer (II; R = H), but are absent from a cyclic polymer. Whereas linear polymers (II; R = H) reacted readily with a chloroform solution of bromine or—in strongly alkaline solution—with *p*-nitrobenzenediazonium chloride, these two substances were inert to both reagents, though coupling with the diazo reagent did take place under conditions known to cause fission of methylene bridges in the *o*- or *p*-position (Ziegler, 1951). The two compounds from octylphenol were similar in chemical properties to their *tetrt.*-butyl analogues.

If a macrocyclic structure is accepted for these substances it still remains to determine the number of phenolic units participating in the large ring. With the compounds from *p-tert.*-butylphenol, consistent values could be obtained by the Rast method, for the molecular weight of the lower-melting substance (hereafter called LBC: low-melting butylphenol cyclic compound), and of the acetate of HBC, the higher-melting substance (itself too insoluble to use). For both HBC and LBC the structure (VI; R = CMe_3), containing four phenolic units and four methylene bridges, was indicated.

The Rast method was less satisfactory with acetates of the two compounds from octylphenol (HOC and LOC). Results were not consistent and were lower than expected. We therefore submitted specimens of HOC, LOC, HBC, LBC, and HOC acetate for *x*-ray crystallographic examination to Dr. D. M. C. Hodgkin, F.R.S., whose valuable assistance is greatly appreciated. Her report is:

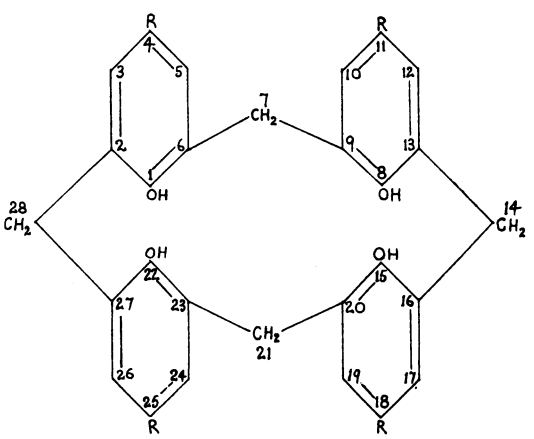
"The preliminary crystallographic data on HOC, LOC, HBC, LBC, and HOC acetate, obtained by E. K. Hunter and M. Mackay, are summarized in Table I. Both HOC and HBC have very complex crystal structures in which the asymmetric units have respectively four and three times the weight required for a tetrapolymer. Formally they admit several solutions for the molecular complexity of these compounds. HOC acetate is rather simpler; the molecule here from the *x*-ray evidence is most probably a 4-polymer (space group $P\bar{1}$), but might be either an 8-polymer, or possibly a 7-polymer (P1), since difficulty was experienced in measuring accurately the lattice constants of the triclinic crystals.

"LOC and LBC both have crystal structures which indicate they are tetrapolymers. Formally, in the case of LOC, the molecule might correspond with a twofold polymer or with an eightfold polymer having a centre of symmetry in the molecule. The latter is, however, not a stereochemically probable solution, and the molecule here almost certainly corresponds with the crystal asymmetric unit. LBC crystallizes in the tetragonal system; here the molecule is proved not only to be a fourfold polymer but also to have either a fourfold or a fourfold alternating axis of symmetry."

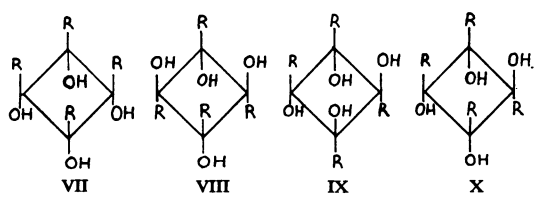
Though they do not establish with certainty the molecular complexity of all the compounds the crystallographic data are consistent with the view that all four condensation products have the general formula (VI). This is reinforced by the Rast molecular weight determinations in the *tert.*-butyl series; those in the *tert.*-octyl series at least refute the possibility, admitted by the crystallographic data, of molecules containing eight phenolic nuclei. We therefore conclude that HOC–LOC and HBC–LBC are pairs of stereoisomerides.

Examination of models indicates that molecules of structure (VI) may assume four different, not readily interconvertible, configurations. These are illustrated diagrammatically (VII to X). Stereoisomerism is possible here because the phenolic nuclei cannot rotate about the bonds joining them to the methylene groups. The molecular symmetry found crystallographically for LBC establishes that this particular molecule has either configuration VII or VIII. It is interesting that LBC forms a clathrate compound with chloroform, from which the solvent is not expelled even after prolonged heating *in vacuo*.

The name *cyclo*tetra-*m*-benzylene is suggested for the ring system with numbering as shown (VI).



VI



VII VIII IX X

Reaction of the Phenolic Products with Ethylene Oxide

Addition of ethylene oxide to the various phenolic products described above was carried out under essentially similar conditions. The product in benzene or toluene, after addition of a little sodium hydroxide as catalyst, was heated with ethylene oxide in a stainless-steel autoclave of 100 ml. capacity. Heating was continued until the ethylene oxide was consumed.

It is highly probable (see, e.g., Miller, Bann, and Thrower, 1950) that the extension of a polyoxyethylene chain under alkaline catalysis involves (1) ionization of the terminal alcoholic group by the alkali, and (2) reaction of the resulting anion with another molecule of ethylene oxide. A phenoxide ion being more easily formed than an alkoxide ion, it may be supposed that phenolic groups will react in preference to alcoholic groups. This is borne out by an experiment in which HOC was heated with ethylene oxide in the presence of a relatively mild base, diethylamine. The product was crystalline, and analysis indicated that reaction under these conditions had ceased after addition of one molecule of ethylene oxide to each phenolic group : that is, the tetra-β-hydroxyethyl ether of HOC was formed. It may therefore be concluded that no free phenolic group remained in our preparations after condensation in the usual manner with ethylene oxide.

The final products (e.g., HOC-20) are often given a number denoting the average number of ethylene oxide units attached to each phenolic group. This figure is an over-all one, based on the consumption of ethylene oxide ; but it is at least possible that some polymerization of ethylene oxide may be initiated by hydroxyl ions as well as by phenoxide ions. Inert polyethylene glycols would then form part of the product and the average length of the chains attached to phenolic residues would be correspondingly shorter. This possibility cannot be excluded as yet. Again, the value given for the chain-lengths is only an average. Individual chains may range rather widely on either side of the mean, and the statistical distribution may vary in different preparations of the same average chain-length. Though these reservations must be made, the "average chain-length" is a useful figure allowing comparison of one preparation with another.

Experimental Procedures

Preparation of Octylphenol-Formaldehyde Polymers.—Recrystallized octylphenol (61.8 g. ; m.p. 83–84°) and hydrated oxalic acid (37.0 g.) were melted together. Formaldehyde (23.1 ml. of 39% w/v aqueous solution) and water (4 ml.) were added and the mixture was stirred and boiled gently under reflux. After 6 hr. toluene (70 ml.) and water (30 ml.) were added ; 10 min. later, heating and stirring were discontinued. Next day the upper layer was separated and the lower layer stirred with a little more toluene. The toluene solution was washed with saturated aqueous $NaHCO_3$ and then with water. The solution was transferred to a distillation flask placed in an oil bath. Solvent was removed at low pressure (water pump), the bath temperature being gradually raised to 160°. Nitrogen was then passed through the capillary leak and the temperature was raised slowly to 220°. Finally the product was heated at 245–255° (air bath) for 1–1¼ hr. at 1–1.5 mm. pressure (nitrogen). The residue (59.2 g.) cooled under nitrogen to a clear, pale amber, brittle resin (D_2 resin). (Found : C, 82.6 ; H, 10.0. Calc. for an infinite linear polymer (II ; R = H ; $n = \infty$) : C, 82.6 ; H, 10.1%).

A resin depleted of lower homologues was prepared by heating D_2 resin (2.97 g.) at 0.01 mm. pressure in an air bath rising rapidly to 320°. To reduce pyrolysis, the time of heating above 280° was kept short (15 min.). The residue (1.9 g.) was an amber resin (D_2A resin).

2:2′ - *Dihydroxy* - 5:5′ - *di*(1:1:3:3-*tetramethyl* - *butyl*) - *diphenyl* - *methane* (II ; R = H ; $n = 0$).—Octylphenol (37.2 g. ; 4 equiv.), 40% w/v aqueous formaldehyde (3.40 ml. ; 1 equiv.) and hydrated oxalic acid (22.0 g.) were stirred under reflux in a bath at 120–130° for 1 hr., then at 110–115° for a further 5 hr. Toluene (40 ml.) and water (20 ml.) were added, and refluxing and stirring were continued for 45 min. Next day the toluene solution was separated, washed neutral with water, and evaporated at low pressure. Excess octylphenol was removed by distillation at 0.5 mm. from a bath at 200° ; the product (12.3 g. ; 64%), a pale yellow viscous oil, then distilled as the bath temperature was raised to 280°. Crystallization of the distillate from light petroleum then gave the *dicyclic phenol* as colourless prisms, m.p. 152–153° (Found : C, 82.3 ; H, 10.0 ; M(Rast), 450. $C_{29}H_{44}O_2$ requires C, 82.1 ; H, 10.4% : M, 424).

2:6-*Bishydroxymethyl* - 4 - (1:1:3:3 - *tetramethylbutyl*) - *phenol* (V).—Octylphenol (15.0 g. ; m.p. 84°), aqueous formaldehyde (150 ml. of 40% w/v) and a solution of NaOH (4.5 g.) in water (18 ml.) were shaken together and kept at room temperature for a week. The filtered solution was diluted to 350 ml., acidified with acetic acid, and extracted with ether (3 × 100 ml.). The ether extracts were washed successively with water, dilute $NaHSO_3$, and water ; filtered, and concentrated. The residual violet gum was dissolved in methanol (15 ml.). Water (15 ml.) and light petroleum (b.p. 60–80° ; 15 ml.) were added and the mixture, after shaking, was left at 0°. Crystallization was best allowed to proceed for a long time (two months). The clustered colourless rhombs were collected and recrystallized from light petroleum (b.p. 60–80°) giving the *bishydroxymethylphenol* (9.95 g.) in the stable needle form, m.p. 76–77°. A specimen was analysed after three further crystallizations and drying over phosphoric oxide (Found : C, 72.6 ; H, 9.7. Calc. for $C_{16}H_{26}O_3$: C, 72.2 ; H, 9.8%). The unstable rhombic form, m.p. 71°, could be obtained by allowing a dilute solution in light petroleum to stand for a few days in a lightly corked flask. This form is apparently the one obtained previously (Sherman, 1938).

2:6 - *Di*[2 - *hydroxy* - 5 - (1:1:3:3 - *tetramethyl* - *butyl*) - *benzyl*] - 4 - (1:1:3:3 - *tetramethylbutyl*) - *phenol* (II ; R = H ; $n = 1$).—The above bishydroxymethylphenol (5.01 g.), octylphenol (15.6 g.), and trichloroacetic acid (0.6 g.) were melted, shaken thoroughly, and heated at 90–100° (bath) for 3½ hr. Excess octylphenol was removed by distillation up to 200°/0.01 mm. and the residue (in three batches) was distilled from bulbs at 300–330° (air bath)/0.01 mm.,

a small fore-run of octylphenol being rejected. The clear pale yellow distillate (7.95 g.) was redistilled at 300° (bath)/0.005 mm.; on cooling it formed a brittle almost colourless resin (Found : C, 82.0; H, 10.4; M (cryoscopic in phenol), 629, 614, 625. $C_{44}H_{66}O_3$ requires C, 82.2; H, 10.3%; M, 642). This *tricyclic phenol* (II; R = H; n = 1) eventually crystallized from methanol; after recrystallization twice from methanol-water and once from methanol it formed colourless needles, m.p. 95–98° (clear at 105°). The analytical sample was dried over phosphoric oxide *in vacuo* (Found : C, 82.3; H, 10.0%).

Crude Tetracyclic Phenol (II; R = H; n = 2).—The dicyclic phenol (5.43 g.; prepared as above) was dissolved in acetic acid (50 ml.), and formaldehyde (0.245 ml. of 40% w/v) was added. The clear solution was saturated with dry HCl. After four days the mixture was poured into water (500 ml.) and the turbid liquid was extracted with light petroleum (b.p. 60–80°). The petrol solution was washed with water and evaporated. The product was distilled in bulbs up to 260° (bath)/0.01–0.05 mm., to remove most of the dicyclic phenol. The residue (2.70 g.) was a pale yellow resin (D_{10} resin).

Crude Pentacyclic Phenol (II; R = H; n = 3).—The dicyclic phenol (4.78 g.), the bishydroxymethylphenol (0.75 g.), and trichloroacetic acid (0.3 g.) were dissolved in acetic acid (30 ml.). The solution after being heated at 90–100° for 5½ hr. was left for several days at room temperature. The mixture was then worked up as described in the previous experiment; the residue (3.03 g.) after removal of excess dicyclic phenol was a pale yellow resin having a greenish fluorescence.

Molecular Distillation of Crude Tetracyclic and Pentacyclic Phenols.—The still was based on the design of Smith and Matalon (1950). In one experiment crude tetracyclic phenol (D_{10} resin; 7.62 g.) was distilled in two batches. After a few minutes at 280°/0.002 mm. the still was cooled and a crust of dicyclic phenol and splashed material were washed off the condenser. The distillation (at 270–290°) was then continued for 2¼ hr. The distillate (2.53 g.) was redistilled (3¼ hr. at 250–260°/0.001 mm.) giving a brittle amber resin (D_{10}A resin). The residue in the still from the first distillation is termed D_{10}B resin.

The crude pentacyclic phenol (2.74 g.) was distilled similarly at 300–310°/0.002 mm. for 2¼ hr., then 3 hr. The combined distillate (0.3 + 0.1 g.) formed a clear amber resin with a green fluorescence (D_{12} resin).

Countercurrent Distribution of Crude Tetracyclic Resins D_{10} and D_{10}A.—A 12-tube all-glass Craig apparatus (Craig, 1950) was used. The solvents were 94% v/v aqueous ethanol and ligroin (b.p. 88–101°) mutually saturated at the cold-room temperature (1° ± 1°) at which the distributions were effected. The D_{10} resin (1.14 g.) was dissolved in the first upper layer and the solution was passed in the known manner through the apparatus, lower layer volumes being 92 ml. and upper layers 20 ml. The process was stopped when the original upper layer had been equilibrated in the 12th and last tube. The ultraviolet absorption at 287 mμ of each upper and lower layer was determined after suitable dilution. Thereafter, the total contents of the first five tubes were combined and the solute (0.64 g.) redistributed as before, but with an upper layer volume of 30 ml. The solute (0.43 g.) from the first five tubes was then redistributed, using an upper layer volume of 50 ml. The results of these three distributions are shown in Figs. 1, 2, and 3. Two successive distributions were carried out in a similar manner with D_{10}A resin (Figs. 4 and 5). It is evident, even with the final distributions, that the partition ratio varies continuously from tube to tube. (The implicit assumption that optical

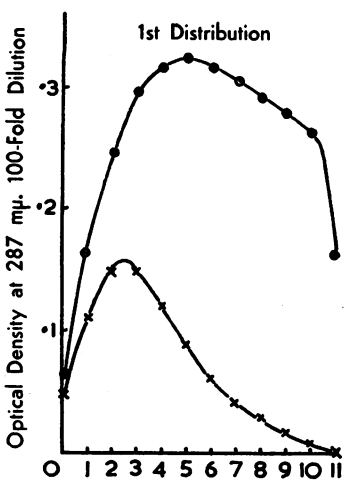


FIG. 1

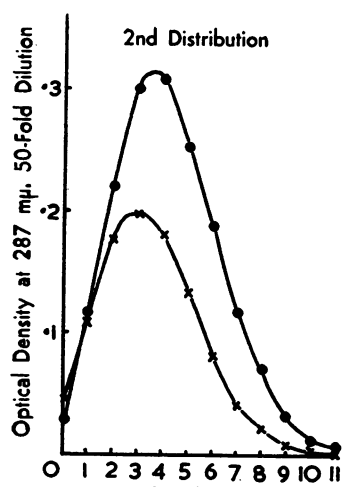


FIG. 2

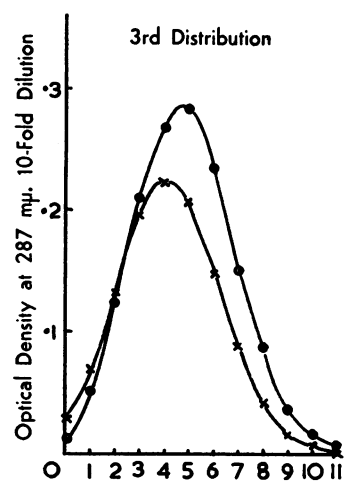


FIG. 3

FIGS. 1, 2, and 3.—Countercurrent distributions of crude tetracyclic compound. Ordinates: optical density at 287 mμ. Abscissae: tube number. ⊙, upper layers; ×, lower layers.

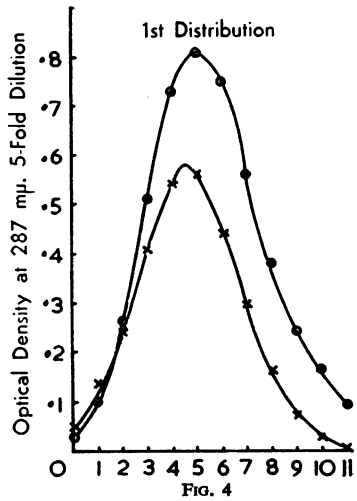


FIG. 4

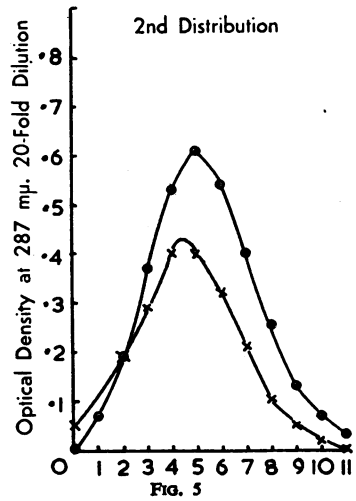


FIG. 5

FIGS. 4 and 5.—Countercurrent distributions of distilled [tetracyclic compound. Ordinates: optical density at 287 mμ. Abscissae: tube number. ○, upper layers; ×, lower layers.

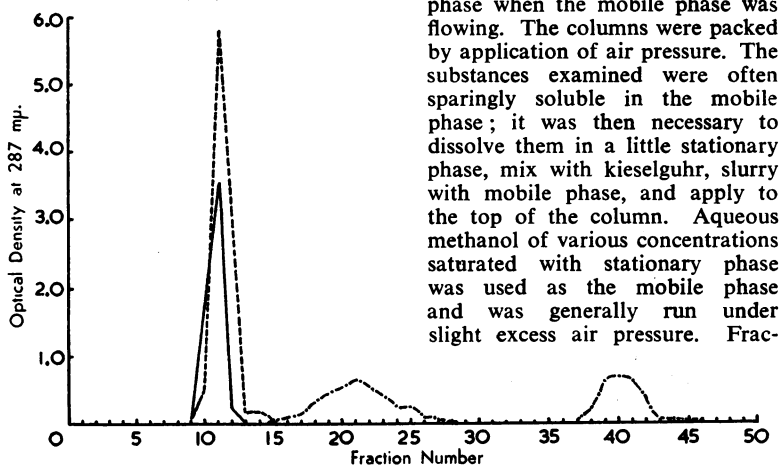


FIG. 6.—Partition chromatography of dicyclic and tricyclic compounds. Ordinate: optical density at 287 mμ. Abscissa: fraction number. ——— Dicyclic (2 mg.); silane-treated hyflo (12.5 g.); *n*-octane (2.0 ml.); 92.5% v/v aq. methanol. - - - - Tricyclic (3 mg.); silane-treated hyflo (12.5 g.); *n*-octane (2.0 ml.); 92.5% v/v aq. methanol. -.-.-.-. Dicyclic (1.5 mg.)+tricyclic (1.0 mg.); silane-treated hyflo (6.25 g.); C_{12} hydrocarbon (5.0 ml.); 70% v/v aq. methanol changed to 96% after collecting fraction 34.

density is proportional to concentration, i.e., that Beer's law holds for this solute in each solvent at the range of concentrations taken, was verified by experiment.)

Reversed-phase Partition Chromatography of Resin Mixtures.—Kieselguhr (Johns-Manville "Hyflo Super-Cel"), made water-repellent by treatment with dichlorodimethylsilane (Howard and Martin, 1950), was used to support the stationary phase. Among the hydrocarbons tried as stationary phase, *n*-octane, ligroin (b.p. 88–101°), and a C_{12} hydrocarbon cut kindly supplied by Shell Chemicals, Ltd., were found useful; especially the last, which showed very little leakage of stationary phase when the mobile phase was flowing. The columns were packed by application of air pressure. The substances examined were often sparingly soluble in the mobile phase; it was then necessary to dissolve them in a little stationary phase, mix with kieselguhr, slurry with mobile phase, and apply to the top of the column. Aqueous methanol of various concentrations saturated with stationary phase was used as the mobile phase and was generally run under slight excess air pressure. Frac-

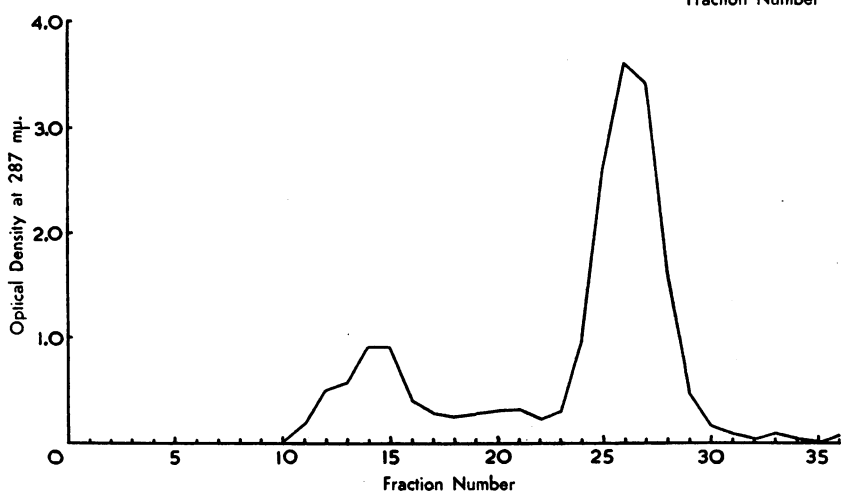


FIG. 7.—Partition chromatography of tetracyclic compound. Ordinate: optical density at 287 mμ. Abscissa: fraction number. ——— CCMD (8 mg.); silane-treated hyflo (12.5 g.); *n*-octane (3.75 ml.); 92.5% v/v aq. methanol. CCMD =contents of tube 5 in 2nd countercurrent distribution (Fig. 5) of twice molecularly distilled crude tetracyclic compound.

tions of 4 ml. volume were examined for absorption at 287 mμ.

A mixture of dicyclic and tricyclic phenols was not separated when 92.5% methanol was used, but 70% methanol gave good separation (Fig. 6). Analysis of the tetracyclic phenol (Fig. 7) showed that this was not free from lower homologues (dicyclic and perhaps tricyclic) even after two molecular distillations followed by two countercurrent distributions. The crude pentacyclic phenol (Fig. 8) was similarly shown to contain the tetracyclic phenol as well as lower homologues. Chromatography of D_2 resin gave a curve (Fig. 9) with successive peaks attributable to di-, tri-, tetra-, penta-, and hexacyclic phenols. The final peak in Fig. 9, obtained by substituting ethanol for methanol, is probably due to mixed higher homologues. A resin obtained by two successive precipitations of D_2 resin with methanol showed the expected lower proportion of dicyclic and tricyclic constituents (Fig. 9); a resin (D_{25}) obtained by repeated precipitation of D_2 resin with methanol showed no peak corresponding to polymers with less than seven phenolic nuclei.

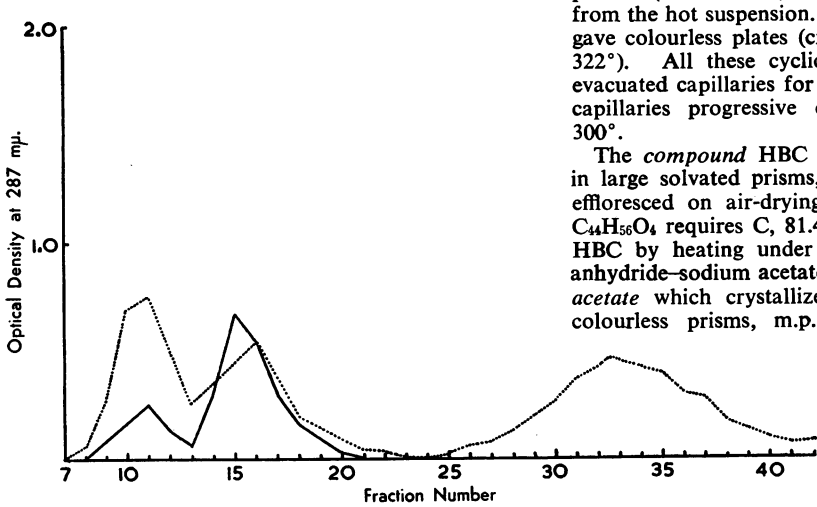


FIG. 8.—Partition chromatography of tetracyclic and pentacyclic compounds. Ordinate: optical density at 287 mμ. Abscissa: fraction number. ——— Crude tetracyclic compound (2 mg.). ..... Crude pentacyclic compound (5 mg.) applied in stationary phase. Each column contained silane-treated hyflo (12.5 g.) and C_{18} hydrocarbon (6.25 ml.). Mobile phase 96% v/v aq. methanol.

1:8:15:22 - *Tetrahydroxy* - 4:11:18:25 - *tetra* - tert. - *butyl* - cyclo - *tetra* - m - *benzylene* (VI; R = $C(CH_3)_3$).—The method of Zinke and Ziegler (1944) was modified, Dowtherm being used in place of linseed oil; *p-tert.*-butylphenol (10 g.) gave 3.2 g. crude product. This was boiled with acetic acid for half an hour; the insoluble residue was collected, dried, and boiled with toluene (30–50 ml.). The insoluble white powder (crude HBC; 1.6 g.; m.p. 380°) was collected from the hot suspension. Concentration of the filtrate gave colourless plates (crude LBC; 0.7 g.; m.p. 320–322°). All these cyclic compounds were sealed in evacuated capillaries for m.p. determination: in open capillaries progressive decomposition set in above 300°.

The *compound* HBC crystallized from chloroform in large solvated prisms, m.p. 380° (decomp.), which effloresced on air-drying (Found: C, 81.9; H, 8.8. $C_{44}H_{56}O_4$ requires C, 81.4; H, 8.7%). Acetylation of HBC by heating under reflux for 2 hr. with acetic anhydride–sodium acetate gave, on cooling, *HBC tetra-acetate* which crystallized from acetic anhydride in colourless prisms, m.p. 332–333° (decomp.). The substance was dried at 140° in high vacuum for analysis, but even so retained solvent of crystallization: when a toluene solution of the crystals was washed with water, the water became acidic (Found: C, 73.3, 73.2; H, 7.5, 7.8. $C_{52}H_{64}O_8$, $C_4H_6O_3$ requires C, 73.2; H, 7.6%). Another sample was analysed after crystallization from ethyl acetate-ligroin and drying

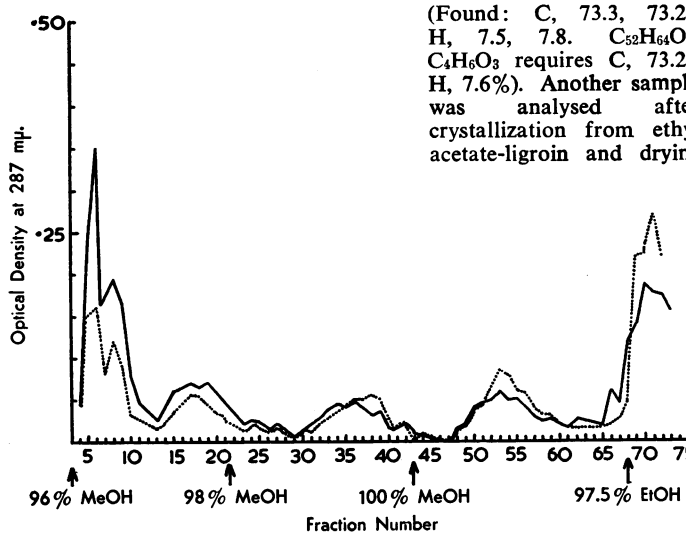


FIG. 9.—Partition chromatography of mixed resins. Ordinate: optical density at 287 mμ. Abscissa: fraction number. ——— D_2 resin (2 mg.). ..... Residue (3 mg.) from double methanol extraction of D_2 resin. Both chromatographed on silane-treated hyflo (6.25 g.) with C_{18} hydrocarbon (3.13 ml.) as stationary phase.

at 140° (Found: C, 76.0; H, 8.1; M(Rast), 789, 832. $C_{52}H_{64}O_8$ requires C, 76.4; H, 7.9%; M, 816).

The *compound* LBC crystallized readily from chloroform in solvated prisms, m.p. 330–332° (decomp.), which still retained chloroform after prolonged heating *in vacuo*. Recrystallization from toluene gave plates, m.p. 320–321° (decomp.). These also contained solvent, but this was expelled on drying in high vacuum at 140° (Found: C, 82.7; H, 8.6; loss at 140°, 13.9%. $C_{44}H_{56}O_4,C_7H_8$ requires C, 82.7; H, 8.7; and contains 12.4% toluene. Found on dried material: C, 81.6; H, 8.7; M(Rast), 628, 623. $C_{44}H_{56}O_4$ requires C, 81.4; H, 8.7%; M, 648). Acetylation, as above, gave *LBC tetra-acetate* which crystallized from acetic acid in solvated prisms, m.p. 247–250° (Found: C, 73.7, 73.5; H, 7.9, 8.0; M(Rast), 879, 899. $C_{52}H_{64}O_8$, $C_2H_4O_2$ requires C, 73.9; H, 7.8%; M, 877). The apparent behaviour of this solute as a single molecule in the Rast determination is probably due to volatilization of acetic acid from the camphor solution.

Neither HBC nor LBC reacted with *p*-nitrobenzenediazonium chloride when dissolved in excess aqueous-alcoholic alkali. However, HBC did give a purple solution in this test when a minimum of alkali was used to dissolve the phenol. Both compounds failed to react with bromine in chloroform solution.

1:8:15:22 - *Tetrahydroxy* - 4:11:18:25 - *tetra* (1:1:3:3 - *tetramethylbutyl*) - cyclo*tetra* - m - *benzylene* (VI; R = $C(CH_3)_2.C(CH_3)_3$).—The following conditions are critical for maximum yields. Octylphenol (40 g.), 3N-NaOH (40 ml.) and aqueous formaldehyde (17 ml.; 33% w/v) were mixed in a jacketed tube and stirred occasionally for 1–2 hr., then heated at the boiling point of acetone for 6 hr. The product was shaken with toluene (80 ml.) and an excess of dilute HCl; the toluene layer was washed with saturated aqueous $NaHCO_3$. Sodium hydroxide (1.8 ml., 3N) was added to the toluene solution and the solvent was removed at lower pressure. The residue was slowly heated during 1–2 hr. at 0.5–1 mm. pressure to 210–220°, and this temperature was maintained for 1¼ hr. The product, a brittle opaque resin (34 g.), was powdered and suspended in boiling acetone (340 ml.). After a few minutes the white insoluble portion was collected and triturated with boiling acetone. The residue (crude HOC; 11–13 g.; m.p. > 300°) was a white powder.

The acetone filtrate was concentrated to half volume and benzene (~ 10 ml.) was added. After 1–3 days crude LOC (1.5–2 g.) was collected; m.p. 220–240°.

Crude HOC dissolved slowly in boiling benzene; after treatment with charcoal, acetone (2–3 vols.) was added and crystallization was allowed to proceed at 37°. The crystallized product was again triturated with boiling acetone and recrystallized first from benzene and finally from benzene-acetone at 37°. The *compound* crystallized in rods, m.p. 315° up to 335° (decomp.), depending on the rate of heating (Found: C, 82.6, 82.5; H, 10.3, 9.8. $C_{60}H_{88}O_4$ requires C, 82.5; H, 10.2%). Acetylation gave *HOC tetra-acetate* which crystallized from acetic anhydride in colourless prisms, m.p. 274.5–275.5° (Found: C, 78.7; H, 8.9; M(Rast), 723. 810. $C_{68}H_{96}O_8$ requires C, 78.4; H, 9.3%; M, 1040). The *tetra-β-hydroxyethyl ether* of HOC was obtained by heating a solution of HOC (2 g.) in toluene (28 ml.) with ethylene oxide (7.9 ml.) and diethylamine (0.15 ml.) in an autoclave at 140–150° for 3½ hr. Slow evaporation of the toluene gave colourless plates (1.4 g.; m.p. 280–285°) which were purified by four recrystallizations from ethyl acetate and then had m.p. 292–293° (Found: C, 77.8; H, 10.1; M(Rast), 1030, 1340. $C_{68}H_{104}O_8$ requires C, 77.8; H, 10.0%; M, 1049).

Crude LOC was purified by two recrystallizations from toluene (charcoal); the *compound* had m.p. 246° (Found: C, 82.9, 82.7; H, 9.9, 9.8. $C_{60}H_{88}O_4$ requires C, 82.5; H, 10.2%). The *tetra-acetate* crystallized from acetic acid in colourless rods, m.p. 259–260° (Found: C, 78.5, 78.5; H, 9.9, 9.4; M(Rast). 826, 874. $C_{68}H_{96}O_8$ requires C, 78.4; H, 9.3%; M, 1040).

Reaction of Phenols with Ethylene Oxide.—A stainless steel autoclave of 100 ml. capacity, fitted with a pressure gauge, was used in all the following experiments. In general, the phenolic substance (0.5–2.0 g.) in toluene was placed in the autoclave and powdered NaOH was added. The autoclave was cooled to 0° and sealed after introduction of liquid ethylene oxide; it was then (sometimes at once, sometimes next day) immersed vertically in an oil-bath at 140°. This

TABLE I
CRYSTALLOGRAPHIC DATA

	a	b	c	β	ρ	Space Group	n	M Asymmetric Unit	M Calc. for Tetrapolymer
HOC ..	23·42	20·68	46·5	102°	1·074	P2/c	4	3,555	876
HOC acetate	a sin γ =15·2	13·6	d_{001} 14·7		1·055	P1 or $P\bar{1}$	1 or 2	1,910 or 955	1,048
HBC ..	20·1	16·9	d_{001} 18·1		1·04	P1 or $P\bar{1}$	1 or 2	3,920 or 1,960	652
LOC ..	11·38	38·5	12·58	100°	1·068	$P2_1/c$	4	884	876
LBC ..	12·72	12·72	14·0		1·09	P4/n	8	$\frac{648}{4}$ (Corr. for solvent)	648

temperature was maintained until no further fall in pressure was perceptible, when the autoclave was cooled and opened. The toluene solution was steam-distilled until frothing became copious; the aqueous solution was roughly neutralized, transferred to a large, weighed flask and evaporated on a steam-bath at low pressure. Water was then added, and the *p*H adjusted, to give a 25% w/w solution at *p*H 7, which was diluted for pharmacological and chemotherapeutic tests with an equal volume of physiological saline. Aliquots of the toluene or water solutions were sometimes evaporated to check whether reaction was complete; the weight of product so determined was always within a few per cent of the theoretical.

TABLE II

ETHYLENE OXIDE CONDENSATION PRODUCTS

Generally, 2 g. of the appropriate phenol, 10 ml. toluene, 0.15 g. NaOH, and the appropriate volume of ethylene oxide were heated at 140° until reaction was complete (usually 1–2 hr.)

Series and Code No.	Chemical Formula
Tr-30 (D_{13})	II; $n=1$; $x=30$
D_2-10·5 (D_{14})	II; $n=0, 1, 2, 3$, etc.; $x=10·5$
M-10 (D_{15})	IV; $x=10$
Tr-10 (D_{16})	II; $n=1$; $x=10$
Di-10 (D_{17})	II; $n=0$; $x=10$
Di-29 (D_{18})	II; $n=0$; $x=29$
M-30 (D_{19})	IV; $x=30$
M-60 (D_{21})	IV; $x=60$
D_2-60 (D_{22})	II; $n=0, 1, 2, 3$, etc.; $x=60$
D_2H-20 (D_{25})	II; $n=5, 6, 7$, etc.; $x=20$

In Table II the ethylene oxide condensation products from compounds of general formula (II; R = H) are listed along with some, prepared from octylphenol, which have the formula (IV). These are compounds not reported in our previous communication.

In Tables III and IV are reported, in somewhat greater detail, the preparation of several products—chiefly from the macrocyclic phenols—which are referred to by code number in the following section.

PHARMACOLOGICAL SECTION

MATERIALS AND METHODS

Surface-active Agents.—For biological testing the agents were made up as 12.5% (w/v) solutions in 0.45% (w/v) saline, adjusted to *p*H 7 and autoclaved. They were usually clear yellow fluids, frothing readily on shaking. The viscosity of the solution varied inversely with the average ethylene oxide chain length of the products; it was marked only in those compounds with less than 15 units of ethylene oxide. For simplicity the surface-active agents will be referred to by series and code number only, but all are described in detail in the earlier part of this paper. They consist of eight series of polyoxyethylene ethers, derived from the following eight intermediates: M (monocyclic-*p-tert.*-octylphenol), Di (linear dicyclic *p-tert.*-octylphenol formaldehyde compound), Tr (linear tricyclic *p-tert.*-octylphenol formaldehyde compound), D_2 (linear polycyclic *p-tert.*-octylphenol formaldehyde mixture; D_2H is a derivative of D_2 but is free from compounds with fewer than 7 phenolic nuclei), LBC and HBC (a pair of stereoisomers of a macrocyclic tetra-nuclear *p-tert.*-butylphenol formaldehyde compound), and LOC and HOC (a pair of stereoisomers of a macrocyclic tetra-nuclear *p-tert.*-octylphenol formaldehyde compound). The final products are referred to their parent intermediate by indicating the average number of molecules of ethylene oxide per chain (HOC-20, etc.).

Tuberculostatic Tests.—The agents were tested against the human virulent strain of *Mycobact. tuberculosis*, H37Rv, in a defined liquid medium containing 0.25% (w/v) albumin (Hart and Rees, 1954).

Chemotherapeutic Tests.—Female mice (18–20 g.) of the albino P strain were infected intravenously by the injection of 0.1 mg. (wet-weight bacilli) of the strain H37Rv, prepared by grinding a fully-grown culture obtained from the surface of Proskauer and Beck's medium. This method produced an acute tuberculous infection, resulting usually in the death within 30 days of all the untreated control mice with

TABLE III

PREPARATION AND CODE NO. OF COMPOUNDS

Series and Code No.	Wt. of Phenol (g.)	Vol. of Toluene (ml.)	Wt. of Catalyst (NaOH) (mg.)	Av. No. Ethylene Oxide Residues/ Phenolic Group	Time at Room Temp. before Heating (Hr.)	Bath Temp. Range (°C.)	Total Time of Heating (Hr.)
D_2-20 (GN/2)	2·00 D_2 resin[1]	8	100	20	<3	140–150	1·3
D_2-27½ (JF/1)	2·00 D_2 ,, [1]	28	100	27·5	16	140–150	0·9
D_2-60 (GN/87)	2·00 D_2 ,, [1]	13	85	60	<1	140–160	2·6
D_2-60 (GN/112)	2·00 D_2 ,, [1]	13	105	60	<1	135–145	2·0
D_2-90 (GN/99)	1·00 D_2 ,, [1]	12	60	90	<1	135–150	2·7
LOC-45 (GN/93)	2·00 LOC	25	102	45	16	140–150	3·0
LOC-60 (GN/90)	2·00 LOC	25	100	60	16	140–150	2·0
LOC-75 (GN/92)	2·00 LOC	25	103	75	16	140–150	2·7
HOC-45 (GN/103)	2·00 HOC	25	115	45	<1	140–150	2·2
HOC-60 (GN/102)	2·00 HOC	25	80	60	<2	140–150	2·7
HOC-75 (GN/104)	1·00 HOC	13	75	75	<1	140–155	2·9

[1] These five samples were all from the same batch of resin.

TABLE IV

PREPARATION AND CODE NO. OF COMPOUNDS

Series and Code No.	Wt. of Phenol (g.)	Solvent Vol. (ml.) a=toluene b=benzene	Wt. of Catalyst (NaOH) (mg.)	Av. No. Ethylene Oxide Residues/ Phenolic Group	Time at Room Temp. before Heating (Hr.)	Bath Temp. Range (°C.)	Total Time of Heating (Hr.)
LBC–15 (GN/14)	0·50	20a	29	15	?16	135–145	5·5
HBC–15 (GN/72)	0·50	25b	40	15	< 1	135–145	5·0
HBC–20 (GN/71)	0·50	25b	36	20	48	135–145	4·5
LOC–15 (GN/76)	1·00	16a	60	15	16	140–160	8·5
LOC–20 (GN/73)	1·00	16a	64	20	16	140–150	3·75
LOC–25 (GN/82)	2·00	33a	92[1]	25	16	135–145 (from r.t.)	2·75
LOC–30 (GN/81)	2·00	32a	100	30	16	135–145 (from r.t.)	2·25
HOC–20 (GN/30)	0·50	7a	14, 27, 40[2]	20	< 1	140–150	3·3, 2·1, 1·6[2]
HOC–20 (GN/57)	0·50	7a	26	20	< 1	140–150	4, 10[3]
HOC–15 (GN/79)	2·00	28a	95	15	< 1–3	140–165	8[4]
HOC–17 (GN/80)	2·00	28a	75	17	3	140–150	4
HOC–20 (GN/68)	2·00	28a	116	20	< 1	140–150	3
HOC–25 (GN/70)	1·00	14a	50	25	< 1	130–150	4

[1] Catalyst and solution heated under reflux before transfer to bomb. [2] At two stages the bomb was left overnight and more catalyst added. [3] Heating in two stages. [4] Bomb left at room temperature overnight after heating for first 2 hr.

gross pulmonary tuberculosis (average survival time 21 days). A standard therapeutic procedure was used, the treated mice (10 or 12/group) being given three intravenous injections of the agent at 25 mg., or at the maximum tolerated dose if less, contained in 0.2 ml. saline, on the 1st, 4th, and 8th days after infection (technical difficulties sometimes made it impossible to give the 2nd or 3rd i.v. dose, or both, and these failures were recorded by marking the mice). The experiments were terminated on the 34th day after infection. The amount of gross tuberculosis of the lung, heart, and kidney was recorded at autopsy. Chemotherapeutic activity of the agent was assessed from the median survival times, and from the amount of macroscopic pulmonary tuberculosis in any animal surviving to the 34th day, in the treated and control groups.

Pharmacological Tests.—Female mice of the albino P strain, 18–20 g. initial weight, were used.

(*a*) *Toxicity.* The approximate acute LD50 of each agent was determined 24 hr. after a single dose administered intravenously. Chronic toxicities were determined (for some of the agents only) by daily subcutaneous doses, or twice weekly intravenous doses, given over a period of 2 weeks.

(*b*) *Lipaemia Production.* Blood was taken from the hearts of mice surviving to the end of the 24-hr. acute toxicity tests. The blood was allowed to clot, centrifuged, and the serum examined with the naked eye for milkiness.

(*c*) *Adrenal Cortical Changes.* The same mice surviving 24 hr. in the acute toxicity tests were killed, and the adrenal glands examined with the naked eye for colour changes in the cortex. It has been shown previously (Rees, 1953), using histochemical tests, that a clear pink adrenal cortex (instead of the normal yellow colour) has no detectable lipid.

RESULTS

In vitro *Activity.*—With the exception of M-10 (D_{15}) and M-30 (D_{19}) none of the present agents, where tested, inhibited the growth of tubercle bacilli at the highest concentrations used (0.2–2.0%, see Table V). Their ability to promote dispersed growth was recorded (see Table V) during these *in vitro* tuberculostatic tests. Some produced dispersed growth, comparable to that obtained with "Triton WR1339," whereas the others had little or no effect, and none was as good a dispersing agent as "Tween 80." There was no correspondence between ability to disperse and chemotherapeutic activity.

Pharmacological Action.—From the results (Table V) it can be seen that, with the exception of the agents of smaller molecular weight (M-10, M-60, Di-10, and Tr-10), these compounds could be given as single intravenous injections to mice in large doses without causing death. On the other hand, many of them had a profound effect on the body lipids, as shown by the production of gross lipaemia and the removal of lipids from the adrenal cortex. These changes, 24 hr. after a single intravenous injection of the agent, are shown in the Table. Although blood cholesterol estimations were not made, an earlier study with somewhat similar products (Cornforth *et al.*, 1951) showed that gross lipaemia was regularly associated with at least a twofold increase in blood cholesterol concentration. More definitive experiments designed to study the production of lipaemia by the present agents have shown that lipaemia remains for at least 36 hr., although it appears

within 30 min.; it also occurs in adrenalectomized mice. The absence of lipaemia production and of stripping of the adrenal cortical lipids by some of the members of the HOC series (e.g., HOC-20) was in sharp contrast to the effect of members of the D_2 and LOC series having similar average ethylene oxide chain lengths. HOC-20 (GN/57) was therefore subjected to a more sensitive test, using the rabbit and giving three intravenous injections (312 mg./kg.) of the agent on alternate days. Blood samples taken 48 hr. after the last injection showed no lipaemia and no rise in blood cholesterol. By contrast, both D_2-20 and the commercial product ("Triton WR1339"), at these doses, produced in rabbits marked lipaemia with the usual associated rise in cholesterol concentration.

Some of the products (including D_2-10.5, D_2-20, D_2-60, and HOC-20) were subjected to chronic toxicity tests in mice. Although in every test the animals gained weight and seemed healthy, microscopical examination revealed liver damage. Considerable individual variation was observed, for, although in more than half there were only a few focal areas of necrosis, the others showed much more extensive liver damage.

Chemotherapeutic Activity.—Table V shows variable activity: an anti-tuberculous effect is

TABLE V
PHARMACOLOGICAL DATA

Surface-active Agent Series and Code No.	Inhibitory Concn. (g./100 ml.)[1] and Type of Growth at Sub-inhibitory Concn.[2]	Acute i.v. Toxicity LD50 in mg. (Single Injection)	Blood and Adrenal Cortex Changes (24 Hr. after Single i.v. Injection)		Chemotherapeutic Activity[5] (Dose, mg.)
			Lipaemia[3] (Dose, mg.)	Loss of Adrenal[4] Cortical Lipid (Dose, mg.)	
M–10 (D_{15})	0·012 g	2·0‡	0 (2)	0 (2)	—
M–30 (D_{19})	0·05 g	7·5	0 (7·5)	0 (7·5)	—
M–60 (D_{21})	>0·5 g	25·0	0 (25)	0 (25)	0 (15)
Di–10 (D_{17})	>0·5 d	5·0‡	0 (5)	0 (5)	—
Di–29 (D_{18})	>0·5 d	15·0	0 (15)	0 (15)	0 (10)
Tr–10 (D_{16})	>2·0 d	5·0‡	0 (5)	0 (5)	—
Tr–30 (D_{13})	>2·0 d	>25·0	+ (25)	+ (25)	0 (15)
D_2–10·5 (D_{14})	>2·0 d	>12·5	+ (12·5)	+ (12·5)	+++ (12·5)
D_2–20 (GN/2)*	>2·0 d	>50·0	+ (15–50)	+ (15–50)	+++ (25)
D_2H–20 (D_{25})	— —	>50·0	+ (25–50)	+ (25–50)	++ (25)
D_2–27·5 (JF/1)	— —	>50·0	+ (50)	+ (50)	0 (25)
D_2–60 (GN/87)	— —	>50·0	0 (50)	0 (50)	0† (25–65)
D_2–60 (D_{22})	>2·0 g	>65·0	0 (25)	0 (25)	0† (25)
D_2–90 (GN/99)	— —	>50·0	0 (50)	0 (50)	0† (25)
LBC–15 (GN/14)	>2·0 d	12·5	+ (10)	+ (10)	0 (10)
HBC–15 (GN/72)	>2·0 d	>50·0	+ (15–50)	+ (25–50)	++ (25)
HBC–20 (GN/71)	— —	>50·0	+ (25–50)	+ (50)	+ (25)
LOC–15 (GN/76)	— —	>50·0	+ (10–50)	+ (20–50)	+++ (25)
LOC–20 (GN/73)	>2·0 d	>50·0	+ (10–50)	+ (25–50)	+++ (25)
LOC–25 (GN/82)	— —	>50·0	+ (10–50)	+ (10–50)	0 (25)
LOC–30 (GN/81)	>2·0 d	>50·0	+ (10–50)	+ (20–50)	0 (25)
LOC–45 (GN/93)	— —	>50·0	+ (10–50)	+ (15–50)	0† (25)
LOC–60 (GN/90)	>0·2 d	>50·0	+ (10–50)	+ (15–50)	0† (25)
LOC–75 (GN/92)	— —	>50·0	+ (10–50)	+ (25–50)	0† (25)
HOC–15 (GN/79)	>2·0 g	>50·0	+ (50)	+ (50)	+++ (25)
HOC–17 (GN/80)	— —	>50·0	+ (50)	+ (50)	++ (25)
HOC–20 (GN/68)	— —	>50·0	0 (50)	0 (50)	+ (25)
HOC–20 (GN/30)	— —	>50·0	0 (50)	0 (50)	+++ (25)
HOC–20 (GN/57)	>0·2 g	>25·0	0 (50)	0 (50)	+++ (25)
HOC–25 (GN/70)	>2·0 g	>50·0	0 (50)	0 (50)	0 (25)
HOC–45 (GN/103)	— —	>50·0	0 (50)	0 (50)	0† (25)
HOC–60 (GN/102)	>0·2 g	>50·0	0 (50)	0 (50)	0† (25)
HOC–75 (GN/104)	— —	>50·0	0 (50)	0 (50)	0† (25)

* This surface-active agent was referred to as D_2 in our earlier papers (Cornforth *et al.*, 1951; Rees, 1953; Hart *et al.*, 1952).

[1] No growth at 2 weeks of *Mycobact. tuberculosis* (H37Rv).

[2] g, granular or partly dispersed growth; d, dispersed growth, comparable to that obtained with "Triton WR1339."

[3] 0: no lipaemia; +: lipaemia.

[4] 0: no loss of adrenal cortical lipoids; +: loss of adrenal cortical lipoids.

[5] 0: inactive; +: low activity, i.e., significant but slight prolongation of median survival time but most of treated mice died of tuberculosis by 34th day. ++: moderately high activity, i.e., considerable prolongation of survival time but more than half treated mice died of tuberculosis by 34th day. +++: high activity, i.e., not more than one treated mouse died of tuberculosis by the 34th day, little macroscopic pulmonary tuberculosis in the surviving mice killed on the 34th day. 0†: "protuberculous," i.e., no chemotherapeutic activity, treated mice dying significantly earlier and with more fulminating tuberculosis than the untreated controls.

‡ Intravascular haemolysis.

confined to the D_2, HBC, LOC, and HOC series and only to those members with an average chain-length of 20 or less ethylene oxide units. The highest activity encountered (+ + +, see Table V) is comparable with that obtained using streptomycin (2 mg. daily) in a similar mouse test. By analysing the protection produced in those mice that received no more than the first intravenous dose of the more active products, it was evident that a single injection given on the first day after infection was as therapeutically effective as the full course.

During this work it was noted that, although some products were inactive (0, see Table), others caused a significant enhancement of the tuberculous infection as compared with the control group. The products showing this effect, which include all members of the D_2, LOC, and HOC series with polyoxyethylene chain-lengths of 45 or more units, are recorded here as "Protuberculous" (0†, see Table).

It has been shown previously (Hart, Long, and Rees, 1952) that a number of our earlier chemotherapeutically active products (D_2-10.5 and D_2-20) depress tuberculin sensitivity in BCG-vaccinated guinea-pigs. Although our newer series were not examined systematically for this property, two therapeutically active representatives (HOC-20 (GN/57) and LOC-20 (GN/73)) were found to possess it.

Discussion

The results now obtained with these eight series of surface-active polyoxyethylene ethers make it possible to define more precisely the structural requirements for antituberculous activity. It is also possible, for the first time, to exclude some of the associated biological properties of these agents as necessary for chemotherapeutic activity.

We can now be sure that the suppression of experimental tuberculosis by the commercial detergent "Triton A20," first reported, was due to the main constituent and not to some unknown chemical contaminant. Since then, this biological property has been found in a variety of allied phenolic condensation products. Some of our newer surface-active agents are derived from phenolic condensation products having a macrocyclic instead of a linear structure. Moreover, the intermediates are pure and crystalline (almost certainly tetracyclic) rather than a mixture, as in the D_2 series. Four such series have been made, two from macrocyclic *p-tert.*-butylphenol (LBC and HBC) and two from macrocyclic *p-tert.*-octylphenol (LOC and HOC) intermediates. Some of the macrocyclic products (HBC-, LOC-, and HOC-) are as active as the linear, and the *p-tert.*-butyl grouping is little less effective than *p-tert.*-octyl.

The previous suggestion (Cornforth *et al.*, 1951) that products derived from the smaller molecular size linear phenolic intermediates (1–3 phenolic nuclei, i.e., M, Di, and Tr series) have no therapeutic activity is confirmed. Activity is not achieved in the linear polynuclear series (D_2) till mixtures containing products with more than three phenolic nuclei is reached; the fact that D_2H-20 (mixture of condensation products with 7 or more phenolic nuclei) has the same order of activity as D_2-20, extends still further the range of active products. It is of interest that the M, Di, and Tr series have a high toxicity in mice and produce considerable haemolysis *in vitro* and *in vivo*. These acute toxic properties decrease with increased molecular size, even with these three series, and are absent from the still larger molecular-sized products from the other five series.

So far we have discussed alterations in the alkylphenol constituent of the molecule in relation to chemotherapeutic activity, but we became aware, from study of the linear series, that the lengths of the ethylene oxide chains were also important. Introduction of the macrocyclic series (HOC and LOC) has defined this factor more precisely. The great activity obtained in these two series by members having an average chain of 15-20* ethylene oxide units was abolished by a small increase to an average chain of 25–30 ethylene oxide units. Moreover, when a chain of 45–75 ethylene oxide units was present, a new biological phenomenon was noted. Although the resulting products showed no chemotherapeutic activity against experimental tuberculosis they were far from inert, for they significantly enhanced the infection (so-called "protuberculous effect"). This unexpected, but consistent, result has been examined in greater detail and will be the subject of a separate communication. It can be concluded that in these three homologous series (D_2, HOC, and LOC) alterations in the lipophilic-hydrophilic balance of the individual products (produced by changing the ethylene oxide chain-length) can profoundly modify the outcome of tuberculous infection. As the lipophilic/hydrophilic ratio decreases, activity passes from antituberculous→inactive→"protuberculous."

* In the HOC-20 series, variations in chemotherapeutic activity have been observed with different batches, a higher activity being associated with smaller scale runs. Investigations by Dr. P. C. Spensley suggest that this is due to a different statistical distribution of ethylene oxide chain-lengths and to somewhat less complete reaction with ethylene oxide in the smaller runs.

The commercial product "Triton WR1339" and our earlier series of linear surface-active agents produced lipaemia, associated with an increase of blood cholesterol and stripping of lipids from the adrenal cortex. At one time it was considered that this phenomenon might be related to chemotherapeutic activity. The high therapeutic activity of some of the HOC series, which produce none of these disturbances in lipid metabolism, now makes it quite clear that these associated effects are not essential. This conclusion confirms our previous (unpublished) observations that: (*a*) smaller doses of D_2-20, insufficient to produce lipaemia and stripping of adrenal lipids, though still raising the level of blood cholesterol, were therapeutically effective; (*b*) a high level of blood cholesterol, maintained by feeding a cholesterol/cholate mixture to mice, did not protect against the standard tuberculous infection.

These products do not appear to be directly antibacterial, for none of those therapeutically active inhibits the growth of tubercle bacilli, even at high concentrations, *in vitro*. Neither has it been possible to detect a directly tuberculostatic substance in the blood or tissue fluids of treated animals. We have thus been led to consider that these agents may act primarily through the host. The finding that some products enhance, while others suppress, a tuberculous infection, in itself suggests an effect mediated through the host rather than directly on the tubercle bacillus.

A number of unsuccessful attempts to find such a mechanism have been reported previously (Rees, 1953). One of the most striking effects produced by some of these agents on the host—altered fat metabolism—can now also be excluded as an operative factor in chemotherapy (see above). More recently, Mackaness (1954) has shown that monocytes harvested from animals previously treated with the commercial agent ("Triton WR1339") become altered in some way so that tubercle bacilli that have been ingested by them are partially or completely inhibited in their growth, or even destroyed. The way in which these agents enhance *in vivo* the ability of monocytes to suppress the multiplication of intracellular tubercle bacilli is at present unknown, but these observations strongly suggest the monocyte as a site of action. This suggestion is further strengthened by using a dye readily solubilized by this type of surface-active agent to demonstrate that the agents do enter the monocyte *in vivo* (Lovelock and Rees, 1955).

One property of surface-active substances is to facilitate dispersed growth of tubercle bacilli, but dispersion in the test-tube is not correlated with activity against tuberculosis in the body, since inactive as well as active members of our series produce dispersed growth. Nevertheless, it seems probable that chemotherapeutic activity of these agents is related, in some way, to their surface-active properties. If this idea is confirmed then a new type of antituberculous agent, based on a physical rather than a chemical action, will have been established.

SUMMARY

1. The preparation is described of linear condensation products from *p*-octylphenol and formaldehyde, including homogeneous dicyclic and tricyclic compounds and less pure tetracyclic and pentacyclic compounds.

2. A method of separating mixtures of linear condensation products is described.

3. Two pairs of crystalline macrocyclic condensation products were prepared from *p-tert.*-butylphenol and from *p-tert.*-octylphenol with formaldehyde. These were shown to be, almost certainly, stereoisomeric forms of a macrocyclic structure with four phenolic nuclei.

4. Conditions are described for the condensation of all these products with ethylene oxide.

5. The effect on tuberculous infection and some of the pharmacological properties of the condensation products are given. The products are arranged in eight series.

6. Some members of the series show high chemotherapeutic activity against a standard tuberculous infection in mice, comparable with that obtained using streptomycin.

7. In determining therapeutic activity ethylene oxide chain-length is critical. Maximum activity is obtained with products having an average ethylene oxide chain-length of 15–20 units.

8. As the ethylene oxide chain-length is increased the effect on tuberculous infection changes from antituberculous→inactive→"protuberculous" (average length of 45 units or more).

9. None of the chemotherapeutically active members of the series inhibits the growth of tubercle bacilli, even at high concentrations, *in vitro*. No tuberculostatic substance has been detected in the blood or tissue fluids obtained from treated animals.

10. It is suggested that these agents act indirectly through the host rather than by direct antibacterial action *in vivo*.

11. The possible site and mode of action of these agents is discussed.

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References

Adler, E., Euler, H. von, and Cedwall, J. O. (1942). *Arkiv. Kemi. Min. Geol.*, **15A**, No. 7.

Cornforth, J. W., Hart, P. D'A., Nicholls, G. A., and Rees, R. J. W. (1953). In: Proceedings of the 6th International Congress of Microbiology, Rome, 1953 (in press). Rome: Staderini.

—— —— Rees, R. J. W., and Stock, J. A. (1951). *Nature, Lond.*, **168**, 150.

Craig, L. C. (1950). In: Weissberger, ed., *Technique of Organic Chemistry*, Vol. III. New York: Interscience.

Euler, H. von, Adler, E., Cedwall, J. O., and Törngren, O. (1942). *Arkiv. Kemi. Min. Geol.*, **15A**, No. 11.

Hart, P. D'A., Long, D. A., and Rees, R. J. W. (1952). *Brit. med. J.*, **1**, 680.

—— and Rees, R. J. W. (1954). *J. gen. Microbiol.*, **10**, 150.

Howard, G. A., and Martin, A. J. P. (1950). *Biochem. J.*, **46**, 533.

Hultzsch, K. (1950). *Organische Chemie in Einzeldarstellung.* III. *Chemie der Phenolharze.* Berlin: Springer.

Koebner, M. (1933). *Angew. Chem.*, **46**, 253.

Lovelock, J. E., and Rees, R. J. W. (1955). *Nature, Lond.*, **175**, 161.

Mackaness, G. B. (1954). *Amer. Rev. Tuberc.*, **69**, 690.

Megson, N. J. L., and Drummond, A. A. (1930). *J. Soc. chem. Ind.*, **49**, 253T.; cf. Koebner (1933).

Miller, S. A., Bann, R., and Thrower, R. D. (1950). *J. chem. Soc.*, p. 3623.

Niederl, J. B., and Ruderman, I. W. (1945). *J. Amer. chem. Soc.*, **67**, 1176.

Rees, R. J. W. (1953). *Proc. roy. Soc. Med.*, **46**, 581.

Sherman, R. (1938). M.Sc. Thesis New York Univ. quoted by Niederl, J. B. (1938). *Industr. Engng Chem.*, **30**, 1272.

Smith, C. C., and Matalon, R. (1950). *Nature, Lond.*, **165**, 613.

Solotorovsky, M., and Gregory, F. J. (1952). *Amer. Rev. Tuberc.*, **65**, 718.

Ziegler, E. (1951). *Sci. Pharm.*, **19**, 10.

Zinke, A., Tomio, M., and Lercher, K. (1942). *Ber. dtsch. chem. Ges.*, **75**, 155.

—— and Ziegler, E. (1944). Ibid., **77**, 264.

—— Zigeuner, G., Hössinger, K., and Hoffmann, G. (1948). *Mh. Chem.*, **71**, 438.