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PREPARATION OF ANTITUBERCULOUS POLYOXYETHYLENE ETHERS OF HOMOGENEOUS STRUCTURE

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Abstract—Polyoxyethylene ethers of homogeneous structure have been prepared from a macrocyclic, tetrahydric phenol ("HOC"), and from cholesterol by reaction with monofunctional derivatives of polyethylene glycols. Many practical difficulties had to be overcome in the preparation of these compounds. The effect on experimental tuberculosis of some of these products was compared with that of corresponding polyoxyethylene ethers produced by random polymerization. Several other surface-active compounds were prepared in an exploration of the structural requirements for chemotherapeutic activity.

The chance discovery[1] that a commercial non-ionic surface-active agent (Triton A-20; Röhm and Haas Co., Philadelphia) suppressed experimental tuberculosis in the mouse led to the examination[2] of several preparations of analogous structure as antituberculous agents. Among these, the most satisfactory were derived from a macrocyclic phenol 2 which for brevity‡ is termed HOC. This phenol (2) is one of two stereoisomeric substances obtained from 4-(1,1,3,3-tetramethylbutyl) phenol (1) by alkali-catalysed condensation with formaldehyde under strictly controlled conditions. The antituberculous agents themselves were made from this phenol by alkali-catalysed condensation with ethylene oxide; when the correct proportions of ethylene oxide were used, the products (3) were water-soluble, non-toxic, and highly active and protective against experimental tuberculosis. A further advantage, not shared by most preparations from other phenols, was the absence of lipaemia following injection.

Antituberculous activity of this type is not confined to polyoxyethylene ethers of phenols; even the presence of a ring is not essential. Thus ethylene oxide condensation products from cholesterol and from 2,2-bis-octadecylpropane-1,3-diol (4) showed antituberculous activity of a lower order. The preparation of these products is reported here. However, numerous other alcohols and polyols containing lipophilic groups§ failed to give active products on condensation with ethylene oxide.

The most active preparations are obtained when the proportion of ethylene oxide used is just enough to form a water-soluble product. This happens when 45–50 molecules of ethylene oxide are condensed with one molecule of HOC phenol. Since the latter has four OH groups, the four resulting polyoxyethylene ether chains have an average length of 11–12½ units, and the product is then called, e.g., HOC-12½ EO (3a). If this average chain length is increased to about 25 units, antituberculous activity is lost; and preparations with still longer chains (45 units upwards) actually accelerate[2] the course of experimental infection. The same progressive change has been shown to occur with polyoxyethylene ethers of other phenols,[2] and is now also reported to be exhibited by polyoxyethylene ethers of cholesterol and of 2,2-dioctadecylpropane-1,3-diol.

Metabolic studies[3] of active preparations labelled, severally, with ^{14}C in the macrocyclic nucleus and in the polyoxyethylene ether chains showed that the drug is remarkably persistent in mice after injection. No radioactivity was detectable in the expired carbon dioxide after injection of material labelled in the polyoxyethylene ether chains; an indication that no significant degradation of these chains occurred *in vivo*.

The condensation of HOC phenol with ethylene oxide is necessarily a complex sequence of reactions. From earlier work with phenol[4] one might expect that all four phenolic OH groups would react with one molecule each of ethylene oxide

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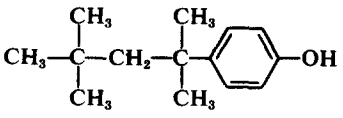
‡The systematic name is 25,26,27,28-tetrahydroxy-5,11,17,23-tetra(1,1,3,3-tetramethylbutyl) pentacyclo [19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]-octacosa-1(25),3,5,7(28),-9,11,13(27),15,17,19(26),21,23-dodecaene. (Ring index No. 6485).

§These substances included 5α-cholestan-3β-ol, cholest-4-ene-3,6-diol, cholest-5-ene-3,4-diol, cholestane-3,5,6-triol, 2-n-octadecylpropane-1,3-diol, 2-n-tetradecylbutane-1,4-diol, batyl alcohol and 2-n-heptadecyl-n-octadecan-1-ol.



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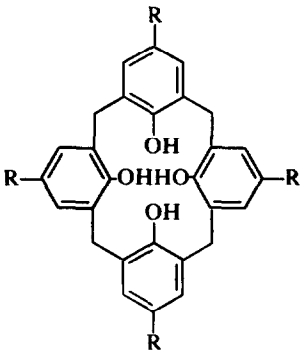
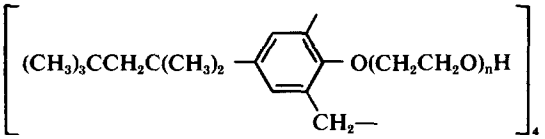
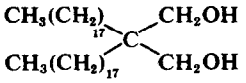


2: R = $C(CH_3)_2CH_2C(CH_3)_3$





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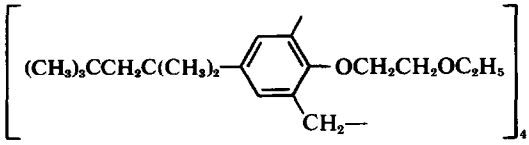
3a: n = 12½ (average)
b: n = 11½ (average)
c: n = 6
d: n = 10
e: n = 1



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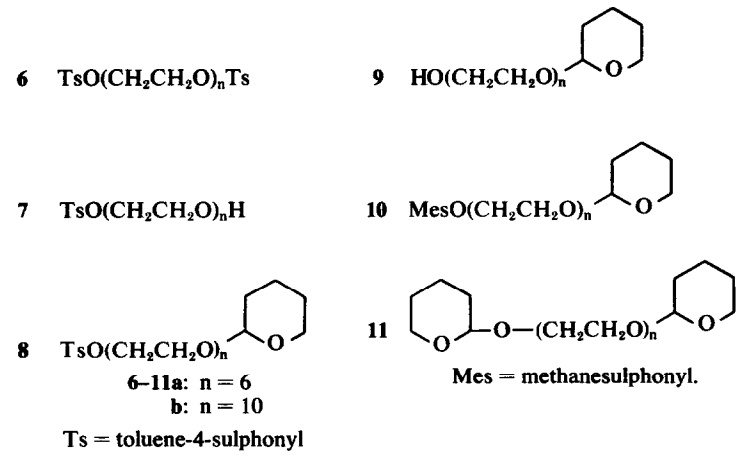
before significant further extension of the chains occurred; and there is confirmation of this view in the isolation[2] of a tetra-2-hydroxyethyl ether of HOC phenol by reaction with ethylene oxide in the presence of a more weakly basic catalyst (diethylamine). With phenol, extension of the polyoxyethylene ether chain has been shown[4] to give a distribution of chain lengths fairly close to the Poisson distribution deduced by Flory[5] for polymerizations of this type. Assuming that this is also true of HOC phenol—each of the four chains growing essentially at random after the addition of the first ethylene oxide unit—it follows that a preparation such as HOC-12½ EO (**3a**) is composed of an extremely large number of molecular species, and that the proportion of molecules having four equal chains, all of about the average length, is quite small. Factors tending to distort the random pattern of condensation, by encouraging the growth of some chains more than that of others, simply increase this lack of homogeneity. It was therefore possible that relatively few of the molecules present in a specimen of HOC-12½ EO were actually effective against experimental tuberculosis, and that a preparation containing a higher proportion of these molecules might be considerably more active.

The principal object of the work reported here was to prepare polyoxyethylene ethers of HOC in which all chains were of equal and definite length, in order to compare the antituberculous activity of these homogeneous substances with that of the products of random condensation.

The phenolic OH groups in HOC phenol are sterically hindered: all of them are *ortho*-disubstituted, and there is probably additional crowding due to the configuration of the molecule. It was therefore to be expected that attachment of preformed polyoxyethylene ether chains to these OH groups would be difficult: one end of the chain would have to be provided with an activating group and this arrangement must necessarily occupy more space than, e.g., an ethylene oxide molecule. Moreover, molecular aggregation would be apt to hinder the later stages of the alkylation. As will be seen, considerable effort was necessary to overcome these difficulties. Fortunately, the presence of free phenolic groups in partially alkylated preparations is readily detected by ultraviolet spectroscopy. In N-methanolic potassium

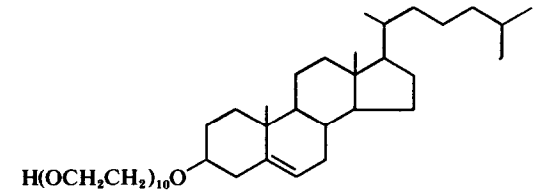


hydroxide, any free phenolic groups give phenoxide ions absorbing strongly at 300 mμ, whereas the phenolic ethers in this series show a characteristic double peak around 270–280 mμ either in neutral or in alkaline ethanol.

First, the feasibility of the chosen method was demonstrated by a successful preparation of the tetra-(2-ethoxyethyl) ether (5) from HOC phenol by successive treatment in benzene with potassium t-butoxide and 2-ethoxyethyl toluene-4-sulphonate. The product (5) was a crystalline substance; m.p. 128–129°. Hexaethylene and decaethylene glycols were then prepared, essentially by the methods of Perry and Hibbert,[6] and the problem of obtaining monofunctional derivatives of these glycols was attacked. Inevitably, mixtures of unreacted glycol with mono- and disubstituted derivatives had to be separated, and this was done in general by combining solvent partition methods with the formation of calcium chloride complexes from components having a free OH group. The reference substances (6, n = 2 and 3) were used to calculate the extinction at 225 mμ of the tosyl group, and thence the expected extinction of the monotosylates (7).

The monotoluene-4-sulphonate (7a) of hexaethylene glycol was separated thus from glycol and disulphonate (6a) in the reaction product from equimolecular amounts of the glycol and toluene-4-sulphonyl chloride. Reaction of this substance with HOC phenol by the method used to prepare 5 gave a product which persistently showed free phenolic OH. Protection of the free OH group in the monotosylate by formation of the tetrahydropyranyl ether (8a) increased the extent of reaction with HOC phenol but still did not give complete reaction.

It had become evident that for this reaction and for the predictably more difficult reaction with a decaethylene glycol derivative, everything possible would have to be done to eliminate competing nucleophilic groups and to facilitate reaction with



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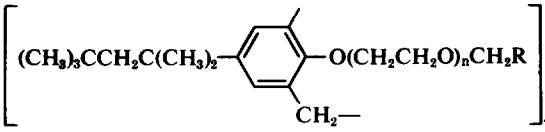
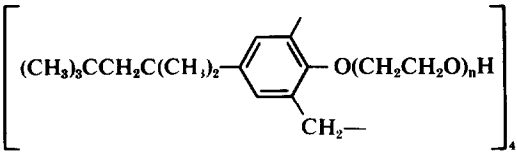


13a: n = 11½ (average); R = CH_2OCH_3
b: n = 11½ (average); R = CO_2H



14: n = 30 (average)

the last, hindered phenoxide ions in the reaction mixture. An apparatus (Fig 3, Experimental) was designed which permitted removal of t-butanol (formed by reaction of potassium t-butoxide with the HOC phenol) by co-distillation with rigorously dried benzene in a stream of dry nitrogen. This technique appeared to be better than the use of phenyl-lithium or of sodium-potassium alloy instead of potassium t-butoxide. The use of the tetrahydropyranyl group to mask free OH groups in the sulphonate derivatives was continued; but methanesulphonates were substituted for toluene-

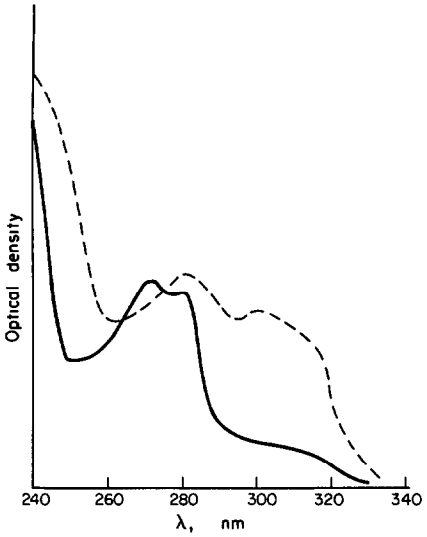


Fig 1. UV absorption (in N KOH in MeOH) of partially and completely reacted HOC phenol: ----- After addition of 4 equivalents of **9a** to HOC and KOBu^t; ——— After addition of 7 equivalents.

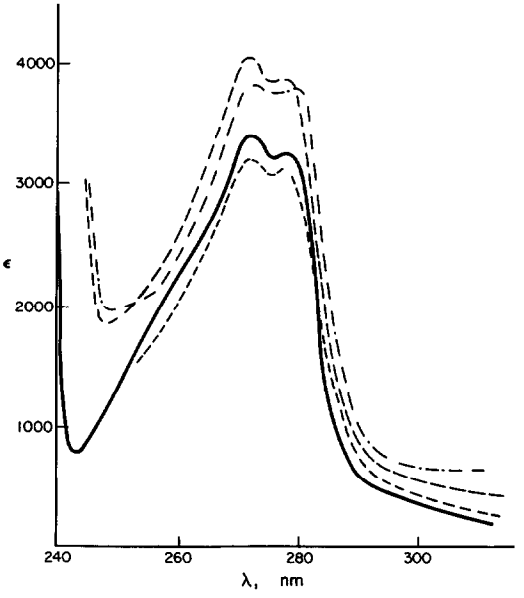


Fig 2. UV absorption (in N KOH in MeOH) of HOC polyoxyethylene ethers: ——— Compound **5**; ----- Compound **3c**; -·-·- Compound **3d**; ------- Compound **3a**.

4-sulphonates, the former being easier to prepare and potentially more reactive in this case because of their smaller size. With these modifications, and some further refinements, success was finally achieved.

Separation of the mixture obtained by reaction between equimolecular amounts of hexaethylene glycol and dihydropyran gave the monoether (**9a**) which was readily converted into the methanesulphonate (**10a**). Reaction of this substance with HOC phenol as outlined above gave a product almost free from phenolic OH. The only method found for removing the last traces of phenolic contaminant was to shake a benzene solution of the product with alkaline potassium ferricyanide. However, this treatment was highly effective; it presumably depends on initial formation of phenoxyl radicals from any free phenolic groups. After removal of the tetrahydropyranyl groups by hydrolysis, and chromatography of the product on alumina, HOC-tetra-(hexaethyleneglycol) mono-ether (**3c**) was obtained as a water-insoluble resin. Some relevant UV absorption spectra are shown in Figs 1 and 2.

For the preparation of **3d**, decaethylene glycol was converted into a mixture of mono- and di-tetrahydropyranyl ethers (**9b** and **11b**) which were separated by solvent partition. The mono-ether (**9b**) was converted into the methanesulphonate (**10b**), which was used to etherify HOC phenol. A final difficulty was encountered in the removal of the tetrahydropyranyl groups by hydrolysis, for 5-hydroxypentanal could not be efficiently removed from the product and, on drying, some tetrahydropyranyl ether was reformed. Oxidation of the hydrolysed solution by Willstätter and Schudel's hypoiodite reagent[7] destroyed 5-hydroxypentanal and after chromatography on alumina HOC tetra-(decaethyleneglycol)-mono-ether (**3d**) was obtained. Its general properties resembled those of the "random" polymer "HOC-12½ EO". Its analysis and its UV and IR spectra were as expected but other criteria of purity could not be applied to this surface-active substance: for example, the partition ratio between immiscible solvents varied with the concentration. The method of preparation, however, can be assumed to have ensured that polyoxyethylene chains of identical length were attached to all the OH groups of HOC phenol. Cholesteryl decaethyleneglycol mono-ether (**12**) was prepared without difficulty from cholesterol by the same method.

Three other derivatives of HOC phenol were also prepared. Two were derived from random condensation products of the phenol with ethylene oxide. First, HOC-12½ EO (**3a**) was methylated so as to transform virtually all the terminal, primary OH groups to methyl ether groups. Next, the potassio derivative of HOC-11½ EO (**3b**) was treated with ethyl chloroacetate to give, after saponification, an analogue of HOC-12½ EO in which carboxyl groups replaced the terminal primary alcoholic groups. The third derivative (**14**), a condensation product of HOC phenol with glycidol, was prepared in order to see whether a more radical departure from the polyoxyethylene chain pattern was compatible with retention of antituberculous activity. It was necessary to

Table 1. Pharmacological data[a]

Compound	Acute i.v. Toxicity LD_{50} in mg (single Injection)	Lipaemia[b]	Chemotherapeutic Activity[c] (Dose, mg)
HOC-12½ EO (**3a**) "Macrocyclon"	> 50·0	0	+++ (25)
HOC-25 EO	> 50·0	0	0 (25)
HOC-60 EO	> 50·0	0	0† (25)
HOC-tetra (hexaethylene glycol monoether) (**3c**)	> 12·5	0	++ (12 5)
HOC-tetra (decaethylene glycol monoether) (**3d**)	> 12·5	0	++ (12·5)
HOC-12½ carboxylic acid (**13b**)	> 25·0	0	+++ (25)
HOC-12½ methyl ether (**13a**)	> 25·0	0	+++ (25)
HOC-polyglyceryl ether (**14**)	> 12·5	0	+++ (12·5)
Cholesterol decaethylene glycol monoether	> 12·5	0	++ (12·5)
Cholesterol-10 EO	18·8	0	± (12·5)
Cholesterol-20 EO	> 12 5	0	0 (12·5)
Cholesterol-60 EO	12 5	0	0† (8·3)
2,2-Dioctadecylpropane-1,3-diol-8 EO	> 25	0	++ (6·25)
2,2-Dioctadecylpropane-1,3-diol-10 EO	4 7	0	0 (3·125)
2,2-Dioctadecylpropane-1,3-diol-16 EO	> 50	+	0 (25)
2,2-Dioctadecylpropane-1,3-diol-64 EO	> 50	0	0† (12·5)
Cholestanol-20 EO	> 25	0	± (9·0)
Cholestanol-30 EO	9·3	0	0 (4·5)
Cholest-4-ene-3,6-diol-7.5 EO	12·5	0	± (8·0)
Cholest-4-ene-3,6-diol-10 EO	9·4	0	± (6·25)
Cholest-5-ene-3,4-diol-6.6 EO	22·5	±	0 (17·5)
Cholestan-3,5,6-iol-20 EO	9·3	0	0 (4·5)
2-Tetradecylbutan-1,4-diol-16 EO	< 1·5	*	*
Batyl alcohol-5 EO	< 3·0	*	*
Batyl alcohol-25 EO	11·5	0	0 (6·0)

[a]Obtained according to the procedures of reference 2.
[b]0: no lipaemia; +: lipaemia.
[c]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 the 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†: "protuberculosis", i.e. no chemotherapeutic activity, treated mice dying significantly earlier and with more fulminating tuberculosis than the untreated controls.
*: Too toxic for test.

add, on average, thirty glyceryl units to each phenolic group in order to get a water-dispersible product.

The toxicities and activities against experimental tuberculosis in mice of some of the surface-active substances mentioned here are summarized in Table 1. It can be seen that although the homogeneous products (**3c**, **3d** and **12**) had activities comparable with those of the standard product of random condensation, no advantage at all is associated with homogeneity of structure; and this evidence suggests that, in the products of random condensation, antituberculous activity of much the same order is shared by a wide range of molecular species. The corresponding variety of lipophilic molecules which can be condensed with ethylene oxide to give antituberculous preparations suggests strongly that this particular biological effect is due to physical rather than chemical properties of these surface-active molecules. Moreover, the high activity both of the methyl ether (**13a**) and of the corresponding tetracarboxylic acid (**13b** show that small modifications at the ends of the hydrophilic chains are without significance, and that *in vivo* oxidation of "HOC-12½ EO" to the tetracarboxylic acid (if it occurs) is not a prerequisite for activity, since this oxidation is presumably blocked in the methyl ether. The antituberculous activity of the glyceryl ether (**14**) shows that even the polyoxyethylene skeleton in the hydrophilic chain is not essential. The lipophilic-hydrophilic balance of the molecule seems to be the most critical factor, though it needs to be reinforced by some resistance to breakdown *in vivo* if activity is to be shown against the relatively slow course of experimental tuberculosis.

EXPERIMENTAL

Diethyl di-n-octadecylmalonate. Diethyl malonate (3·2 g) was added to a soln of NaOEt from Na (0·92 g) in dry EtOH (50 ml). Octadecyl iodide (15·2 g) was added to the boiling mixture and refluxing was continued for 18 hr. The product was separated in the normal manner and distilled to give a fraction (7·1 g), b.p. 254–256°/0 01 mm. After redistillation and crystallization from MeOH, *diethyl di-n-octadecylmalonate* was obtained as white irregular plates, m.p. 50–51°. (Found: C, 77·9; H, 12·6. $C_{43}H_{84}O_4$ requires: C, 77·6; H, 12·7%).

2,2-Di-n-octadecylpropane-1,3-diol. The above ester (2·2 g) in dry ether (30 ml) over 15 min was added to a suspension of LAH (0·5 g) in dry ether (60 ml). The mixture was kept boiling for ½ hr more and then decomposed by dropwise addition of water. The ether soln on evaporation yielded the *diol* (1·2 g); m.p 75–76° unchanged by recrystallization from light petroleum (b.p. 40–60°). (Found: C, 80·2; H, 13·6. $C_{39}H_{80}O_2$ requires: C, 80 6; H, 13·9%).

Condensation of alcohols with ethylene oxide. A typical procedure for alcohols (for phenols see Ref 2) was as follows: the alcohol (2 g) in toluene (25 ml) was treated with t-BuOK (140 mg) or metallic K (40 mg), and when dissolution was complete the appropriate amount of ethylene oxide was added. The mixture was heated in a rotating 100 ml stainless steel autoclave (the capacity of which was sometimes reduced in smaller-scale runs by introduction of a stainless steel cylinder) in a bath at 150° until the pressure fell to a constant value. The contents of the autoclave were neutralized by addition of a few drops of AcOH, the toluene was removed at low pressure, the residue weighed and dissolved, when possible, in water or physiological saline to give a 25% w/v solution for biological testing.

HOC tetra-ethoxyethyl ether. t-BuOK (4·52 g; 20 mmoles) was added to a soln of HOC phenol (4·4 g; 5 mmoles) in dry benzene (50 ml) and the mixture warmed and stirred until homogenous. Then ethoxyethyl toluene-*p*-sulphonate[8] (10 g; 40·9 mmoles) was added and the mixture stirred for 4 hr and left overnight.

The mixture was diluted with water and shaken with ether, the benzene-ether portion washed with water, and dried ($MgSO_4$). Removal of the solvent gave a crude product (8·8 g) which was crystallized from acetone-MeOH twice, m.p. 47–49°, 2·6 g (45%) containing solvent of crystallization. It was dried *in vacuo* at 150–170° and the temp lowered slowly to allow the material to crystallize, giving HOC *tetra-ethoxyethyl ether* (**5**) m.p. 128–129°. (Found: C, 78·3; H, 10·2. $C_{75}H_{120}O_8$ requires: C, 78·5; H, 10·4%); λ_{max} 272, 278 mμ; ε 3,400, 3,105. The spectrum was unchanged in N KOH in MeOH.

Diethylene glycol ditoluene-4-sulphonate. A soln of toluene-4-sulphonyl chloride (80 g) in dioxane (100 ml) was added in small portions with shaking, to a soln of redistilled diethylene glycol (20 g) and NaOH (20 g) in water (100 ml). The mixture was shaken mechanically for 2 hr and left overnight. The product was extracted with benzene, the benzene extract washed with water, dil Na_2CO_3 aq and water, dried ($CaCl_2$) and evaporated to dryness. The residue was crystallized from benzene-ether (39·7 g) m.p. 86–87° (lit.[9] m.p. 88–89°). (Found: C, 51·9; H, 5·24; S, 15·4. Calc. for $C_{18}H_{22}O_7S_2$: C, 52·2; H, 5·35; S, 15·5%); λ_{max} 225 mμ, ε 23,700.

Hexaethylene glycol. Diethylene glycol (900 g 8 5 moles) was added from a dropping funnel to a stirred soln of Na (92 g 4 moles) in dry MeOH (700 ml) in a 3-necked flask fitted with a vacuum-sealed stirrer. The flask was heated in an oil bath and the MeOH was distilled off under vacuum, until a temp of 110° and 12 mm pressure was reached. The flask was then filled with dry N_2 and allowed to cool to 60°, when the mixture began to solidify. 2,2′-dichlorodiethyl ether (286 g 2·0 moles) was added slowly through the dropping funnel while stirring, the flask heated to 95° (bath) and 1 ml portions removed from time to time to test for residual alkali. When the mixture was neutral, NaCl was filtered off and washed with EtOH, the combined filtrate and washings were distilled to remove EtOH and diethylene glycol (b.p. 130°/3 mm). The distillation residue was filtered free of more precipitated salt and distilled in an all-glass wide path distillation apparatus giving hexaethylene glycol b.p. 140–180°/0·01 mm (226 g), redistilled, b.p. 142–162°/0·03 mm (191 g 36% yield). (Found: C, 51·3; H, 9·27. Calc. for $C_{12}H_{26}O_7$: C, 51·1; H, 9·29%). With all these polyethylene glycols, the distillation temp at low pressure varies greatly with the rate of distillation.

Decaethylene glycol This was prepared by the method of Perry and Hibbert from diethylene glycol and hexaethylene glycol dichloride.[6] It was obtained as a pale yellow oil b.p. 230–240°/0 005 mm (56% yield) which solidified, m.p. 26–28°.

Triethylene glycol ditoluene-4-sulphonate. Triethylene glycol was converted to the ditoluene-4-sulphonate ester by the method used above for diethylene glycol. The product was obtained as colourless plates m.p. 81° (lit.[10] m.p. 81–82°) from benzene-ether (1:3). (Found: C, 52·6; H, 5·64; S, 14·26. Calc. for $C_{20}H_{26}O_8S_2$: C, 52·4; H, 5·72; S, 14·0%).

Hexaethylene glycol ditoluene-4-sulphonate. The compound was prepared by the action of toluene-*p*-sulphonyl chloride on a mixture of hexaethylene glycol and pyridine. The *ditoluene-4-sulphonate* (**6a**) was obtained as a pale yellow viscous oil (86% yield), its IR spectrum indicated no free OH groups; it had $E^{1\%}_{1cm}$ 410, λ_{max} 225 mμ (from E_{1cm} for diethylene glycol ditoluene-4-sulphonate, $E^{1\%}_{1cm}$ for this compound is calculated to be 403). (Found: C, 53·0; H, 6·56; S, 10·9. $C_{26}H_{38}O_{11}S_2$ requires: C, 52·8; H, 6·48; S, 10·9%).

Hexaethylene glycol monotoluene-4-sulphonate. Purified toluene-4-sulphonyl chloride (24·8 g 0·13 mole) was added in portions to a mixture of hexaethylene glycol (28 2 g, 0·1 mole), dry, peroxide-free dioxane (50 ml) and dry pyridine (10·1 g, 0·128 mole) and shaken until the acid chloride dissolved and left overnight. Then water was added and the mixture was extracted 3 times with benzene, the combined benzene extracts washed with water, twice with dil HCl and again with water until neutral and dried ($MgSO_4$)

Removal of the benzene gave 22·5 g of crude toluene-4-sulphonates, as a colourless oil. This was redissolved in benzene and shaken with powdered anhyd $CaCl_2$ (20 g) for 30 min, by which time the $CaCl_2$ had become semi-liquid. The benzene was decanted and the $CaCl_2$ complex washed by decantation with fresh dry benzene. The benzene soln was treated with $CaCl_2$ in this way 3 times in all, the last portion being left with the benzene soln overnight. From the benzene mother-liquor was then obtained, by evaporation, hexaethylene glycol ditoluene-*p*-sulphonate (13·3 g 35%) as a pale yellow oil, $E^{1\%}_{1cm}$ 396 at 225 mμ.

The three portions of $CaCl_2$ were dissolved separately in water and extracted twice with benzene. Evaporation

of the benzene gave the following fractions:

1, 6·0 g, $E^{1\%}_{1\,cm}$ at 225 mμ 291;

2, 2·8 g, $E^{1\%}_{1\,cm}$ at 225 mμ 264;

3, 0·4 g, $E^{1\%}_{1\,cm}$ at 225 mμ 345.

From the value found for ethoxyethyl toluene-4-sulphonate, $E^{1\%}_{1\,cm}$ at 225 mμ for hexaethylene glycol monotoluene-4-sulphonate is calculated as 316.

Fractions 1 and 2 were combined, dissolved in benzene and extracted twice with sat $CaCl_2$ aq, and then 3 times with small portions of water. Evaporation of the benzene gave *hexaethylene glycol monotoluene-4-sulphonate* (7; 3·0 g) as an oil (Found: C, 51·8; H, 7·50; S, 7·93. $C_{19}H_{32}O_9S$ requires: C, 52·2; H, 7·39; S, 7·35%) $E_{1\,cm}$ 316 at 225 mμ.

Re-extraction of the combined $CaCl_2$ and aqueous extracts gave more impure mono-tosylate.

Hexaethylene glycol monotetrahydropyranyl ether. Hexaethylene glycol (28·2 g, 0·1 mole) was heated on a steam bath for 30 min with dihydropyran (10·5 g, 0·125 mole, dried over Na) and a few crystals of toluene-4-sulphonic acid, cooled and made just alkaline with a few drops of 3N NaOH, and the mixture extracted with light petroleum (b.p. 60–80°) in a continuous extractor for 4 hr. There remained unextracted glycol (5·76 g, 20%). The light petroleum soln was treated with two successive portions of powdered $CaCl_2$ (50 g and 20 g). From the light petroleum was obtained the *diether* (**11a**) of hexaethylene glycol (14·6 g, 33%). (Found: C, 58·7, H, 9·34. $C_{22}H_{42}O_9$ requires: C, 58·6; H, 9·4%). The IR spectrum showed no trace of OH absorption.

The combined $CaCl_2$ extracts were dissolved in water (800 ml) and extracted 3 times with chloroform. The chloroform extracts were washed with water, dried and the solvent removed, giving the *mono-ether* (**9a**; 16·9 g, 46%) as a mobile yellow oil. A sample distilled at 130°–160°/0·002 mm in a short path distillation apparatus was obtained as a clear colourless oil. The IR spectra of the distilled and undistilled material were identical. (Found: C, 55·5; H, 9·67: $C_{17}H_{34}O_8$ requires: C, 55·7; H, 9·35%). Subsidiary experiments had established that chloroform does not appreciably extract hexaethylene glycol from dilute calcium chloride solns.

Tetrahydropyranyl hexaethylene glycol methanesulphonate (**10a**). Methanesulphonyl chloride (8·5 g, 75 mmoles) and pyridine (6·65 g, 79 mmoles) were mixed in a small flask and cooled in ice, then added to hexaethylene glycol monotetrahydropyranyl ether (13·6 g, 37·5 mmoles) also at 0° and shaken together at this temp for 30 min and left at room temp several hr. Water was added, slowly, with cooling until one phase was obtained and then solid K_2CO_3 until the soln was alkaline. It was then diluted to 500 ml with water and extracted 4 times with chloroform. The combined chloroform extracts were washed with dil HCl aq, twice with water and once with Na_2CO_3 aq, dried ($MgSO_4$) and the solvent removed giving the *ether-ester* (**10a**) as a golden yellow oil (15·10 g, 91·5%). (Found: C, 48·3; H, 8·4; S, 7·04. $C_{18}H_{36}O_{10}S$ requires: C, 48·7; H, 8·17; S, 7·21%).

Decaethylene glycol tetrahydropyranyl ethers. Decaethylene glycol (9·16 g, 2 mmoles) dihydropyran (2·10 g, 2·5 mmoles) and one crystal of toluene-*p*-sulphonic acid were heated together on a steam bath for 30 min, cooled to 10° and neutralized to phenolphthalein with NaOH aq and continuously extracted with cyclohexane. In 4 hr the volume of unextracted material was reduced to about 1/4, the extraction was stopped and from the cyclohexane, by evaporation, was obtained a mixture of tetrahydropyranyl ether and some unchanged glycol as a pale yellow oil (9·3 g). This crude mixture in benzene (400 ml) was shaken with water (400 ml) and the aqueous portion washed twice with 200 ml portions of benzene. Evaporation of the benzene gave *decaethylene glycol ditetrahydropyranyl ether* (**11b**; 4·1 g, 30·5%) as a pale golden oil, n_D^{25} 1·4665. (Found: C, 57·2; H, 9·11. $C_{30}H_{58}O_{13}$ requires: C, 57 5; H, 9·33%).

The aqueous portions from the above extractions were saturated with NaCl and re-extracted 3 times with benzene, the benzene extracts washed once with NaCl aq and dried ($MgSO_4$). Removal of the benzene gave *decaethylene glycol monotetrahydropyranyl ether* (**9b**; 4·7 g, 44%) as a colourless oil, which crystallized on cooling, m.p. 0°. (Found: C, 55·4; H, 9·24. $C_{25}H_{50}O_{12}$ requires: C, 55·3; H, 9·30%). It had been established by subsidiary experiments that decaethylene glycol is not appreciably extracted by benzene from aqueous sodium chloride solutions.

Tetrahydropyranyldecaethylene glycol methanesulphonate. The *ether-ester* (**10b**) was prepared as described for the hexaethylene glycol analogue (**10a**). It was obtained as a pale yellow oil (95% yield). (Found: C, 50·0; H, 8·42; S, 4·76. $C_{26}H_{52}O_{14}S$ requires: C, 50·3; H, 8·45; S, 5·17%).

HOC *tetra* (*Hexaethylene glycol monoether*) (**3c**). t-BuOK soln was prepared by the method of Johnson and Schneider[11] and standardized against 0·1 N HCl. It was transferred by reverse filtration into ampoules and sealed for use as required.

All apparatus (Fig 3) was baked before assembly. Flask A (500 ml) contained dry benzene (200 ml) refluxing over Na. Into flask B (100 ml) was introduced HOC phenol (0·873 g, 1 mmole) and benzene was then run in from flask A, with stirring and heating of flask B on an oil bath, so that the benzene slowly distilled out into the long narrow air condenser. A slow stream of N_2 (dried over $CaCl_2$, soda-lime and P_2O_5) was passed through the reaction flask. Then t-BuOK in t-BuOH (2·7 ml of freshly standardized 1·49 N soln, 4 mmoles) was added and the material in flask B distilled to dryness twice, refilling each time (to about 25 ml) with benzene from flask A. The refractive index of the distillate was followed to indicate the removal of t-BuOH.

A soln of tetrahydropyranyl hexaethylene glycol methanesulphonate was so prepared in dry benzene that 3 ml of soln contained accurately 0·4445 g (1 mmole) of the material. 12 ml of this soln was placed in the dropping funnel and allowed to drop slowly through a layer of magnesium perchlorate into the reaction flask. The colour of the soln turned from straw to deep red. The mixture was kept distilling gently for 3 hr and left overnight.

A sample was taken for examination of the UV spectrum and then more t-BuOK soln (0·675 ml, 1 mmole) was added. When the refractive index of the distillate became constant at n_D^{32} 1·4989 more methanesulphonyl ester (3 ml of soln, 1 mmole) was added and distillation continued for 3 hr. Again the UV spectrum was examined and the process repeated with a 6th and 7th equiv of t-BuOK and methanesulphonyl ester. The UV spectrum of the product now showed almost complete absence of phenolic material.

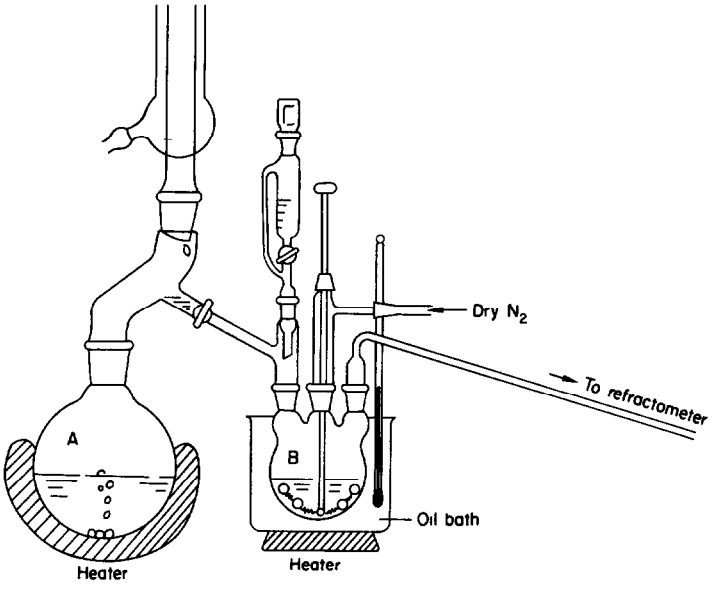


Fig 3. Reaction Apparatus.

The mixture was diluted with light petroleum (b.p. 40–60°) and shaken 3 times with dil NaCl aq to remove excess glycol derivatives, then with alkaline ferricyanide soln (from 7 g $K_3Fe(CN)_6$, 5 g NaOH, 50 ml water), washed with water, and the solvent removed, giving HOC *tetra* (tetrahydropyranyl-*hexaethylene glycol*) *ether* as a viscous resin (1·95 g, 89%). Its UV spectrum (in alkali) showed complete absence of phenolic material.

The crude product (1·85 g) was heated at 100° for 2½ hr in dil HCl, with sufficient EtOH to produce a homogeneous soln, and extracted with chloroform. The chloroform extract was washed with water and dil Na_2CO_3 aq, dried over K_2CO_3 and the chloroform removed, leaving a red gum (1·50 g). This was chromatographed on a column of alumina (40 g) prepared in cyclohexane. The product was eluted with chloroform-MeOH (50:1) giving HOC *tetra* (hexaethylene glycol monoether) **3c** (1·09 g) as a stiff golden resin, insoluble in water. (Found: C, 67·7; H, 9·5. $C_{108}H_{184}O_{28}$ requires: C, 67·2; H, 9·6%); λ_{max} 272 mμ, ε 4,000.

HOC *tetra* (*decaethylene glycol monoether*). This was carried out as when using the hexaethylene glycol derivative, except that heating was more prolonged after each addition of methanesulphonyl ester; thus after 4 mmoles, 8 hr; after the 5th, 9 hr; after the 6th, 36 hr; after the 7th, 24 hr. During these long periods of heating the condenser and adapter in flask B were replaced by a cold finger with a side arm.

The mixture was diluted with benzene at the end of the reaction and the benzene soln was washed with alkaline ferricyanide (5 ml), the aqueous layer was saturated with NaCl to help the separation into two layers, the benzene layer was dried ($MgSO_4$) and the benzene removed giving the tetrahydropyranyl ether of the required product as a pale yellow viscous oil (4·7 g) contaminated with excess glycol derivatives but free of phenolic impurities.

This product dissolved readily in water, and so was heated with N HCl (40 ml) at 60–70° for 2½ hr to hydrolyse the tetrahydropyranyl groups, neutralized with solid Na_2CO_3 and the soln saturated with NaCl. The product which separated was redissolved in benzene and the extract washed with sat NaCl aq, dried, and the benzene removed, giving a viscous orange oil (2·82 g).

Chromatography on alumina (60 g) prepared in ether, eluted material (2·52 g) with chloroform containing up to 2% MeOH. These fractions had identical IR spectra and analysed correctly for the required product, but counter-current partition between 2-butanone and water showed that they were not homogeneous.

A sample hydrolysed again and tested with sodium hypoiodite according to Willstätter and Schudel[7] showed the presence of aldehyde. The combined chromatography fractions (2·31 g) were hydrolysed again, neutralized and diluted to 500 ml with water: 5-hydroxypentanal was oxidized with standard hypoiodite and excess of the reagent destroyed by titration with standard thiosulphate. It was found that the chromatographed material was only 61% hydrolysed.

The material was now chromatographed again; the fractions collected (1·27 g) showed no inhomogeneity in counter-current distribution. The UV and IR spectra were essentially identical with those of HOC-12½ EO. The fractions were combined to give HOC *tetra* (*decaethylene glycol monoether*) (**3d**) as a golden yellow resin. (Found: C, 63·6; H, 9·50. $C_{140}H_{248}O_{44}$ requires: C, 63·8; H, 9·48%). It was soluble in water at room temp at 25% w/w concentration as a mobile soln. The soln became viscous on warming and the compound was precipitated as a gum from the boiling soln.

Cholesterol decaethylene glycol monoether (**12**). Using the same apparatus, t-BuOK (3·35 ml of 1·49 N soln in t-BuOH, 5 mmoles) was added to a soln of cholesterol (1·93 g, 5 mmoles) in benzene; a gel was immediately precipitated. The soln was evaporated to dryness twice, more benzene being added after each evaporation, and a soln of tetrahydropyranyl decaethylene glycol methane sulphonate (3·05 g, 4·9 mmoles) in dry benzene was added slowly from the dropping funnel. Reaction began immediately and the K-salt of cholesterol began to disappear. Heating was continued for 30 min after all the

methane sulphonate was added Next day the product, which was neutral, was filtered free from salts, and evaporation of the solvent gave the crude tetrahydropyranyl ether of the required product as a pale yellow solid (4·45 g).

This was dissolved in a mixture of N HCl (40 ml) and MeOH (10 ml) and hydrolysed for 3 hr, neutralized, and treated with hypoiodite, excess of the reagent being titrated with sodium thiosulphate. The organic material was chromatographed on alumina deactivated with AcOH The fractions eluted with benzene-chloroform mixtures, after being tested for the absence of cholesterol with digitonin, were rechromatographed and then partitioned between benzene and water to remove glycol derivatives. Evaporation of the benzene portion gave *cholesterol decaethylene glycol monoether* as a semi-solid wax (0 25 g). (Found: C, 67·9; H, 10 1. $C_{47}H_{86}O_{11}$ requires: C, 68·2, H, 10·5%). It was soluble in water at 12½% w/w concentration at room temp. Cooling the viscous soln reduced the viscosity, heating it precipitated the material.

HOC-12½ *methyl ether* (**13a**). The polymer (HOC-12½ EO) made from HOC phenol and ethylene oxide with an average polyoxyethylene chain length of 12½ units (5 g) was refluxed in dry MeI, and small portions of dry Ag_2O (prepared by the method of Pearl[12] and dried finally over P_2O_5) were added from time to time over a total of 8 hr After filtering off solids the soln was evaporated to dryness and toluene distilled from the residue to remove traces of water. The IR spectrum of the product showed only a faint band corresponding to OH in the 3 μ region.

The product was treated twice more in the same way with MeI and Ag_2O, until the OH band in the IR spectrum was very faint. The product, in ether, was passed through a 5 cm column of activated charcoal and celite, prepared in ether. The eluant, on evaporation, gave a soft solid, which tended to crystallize in rosettes. It was soluble in water at 12½% w/w conc.

2-C^{14}-acetic anhydride was standardized by converting a portion into C^{14}-acetanilide. The acetanilide was found to have a specific radioactivity of 9·10 mμC/mg; hence the acetyl group had a specific activity of 28·6 mμC/mg. The methylated polymer (3·15 mg) and the radioactive Ac_2O (2·5 ml) were dissolved in dry pyridine (2 ml) and refluxed for 30 min. On cooling, the excess Ac_2O was destroyed with water, the solvent removed, and toluene was distilled from the residue to remove traces of AcOH and water. From the radioactivity of the acetylated polymer (0·053 mμC/mg) it was found that there were 27 methylated end groups to one acetylated group; the methylation reaction was therefore 96·5% complete.

HOC-12½ *carboxylic acid* (**13b**). HOC-11½ prepared in the manner described[2] (6·8 g) in dry benzene (50 ml) was added to powdered K (380 mg) stirred in refluxing benzene (50 ml). The K slowly dissolved forming a brown gelatinous suspension. Ethyl chloroacetate (3 g) was then added and stirring and refluxing continued until the mixture was neutral (2 hr). Removal of the solvent gave a gum, which showed a strong ester absorption and a weak OH band in its IR spectrum.

This product (6·8 g) was refluxed with sat $Ba(OH)_2$ aq (100 ml) for 8 hr; the Ba ions were precipitated with a slight excess of N H_2SO_4, and back titrated with 0·3 N $Ba(OH)_2$ to neutrality. The $BaSO_4$ ppt was removed by centrifugation. The soln was passed through a column of "Zeocarb 215" cation-exchange resin, to remove Ba ions, and the polymer obtained was a yellow wax (4·5 g) on evaporation of the eluant. By dissolving a sample in 0·1 N NaOH and titrating with 0·1 N HCl, it was calculated that carboxylation of the polymer was complete within the limit of error of the titration

HOC *polyglyceryl ether* (**14**). HOC phenol (0·87 g) and t-BuOK (150 mg) were dissolved in a mixture of diethoxyethane (10 ml) and glycidol (8·9 g, freshly prepared) and left at room temp for a few min, then heated slowly until all the diethoxyethane was removed. The resulting polymer was a transparent, pale yellow resin. After neutralization with AcOH it dissolved in water at 25% w/w conc to give a stable, white colloidal soln.

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