THE PREPARATION AND THE ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF SOME SUBSTITUTED BENZYL ALCOHOLS

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A number of substituted benzyl alcohols have been prepared and together with some related, commercially available, compounds have been tested for antibacterial and antifungal properties. The most active inhibitory compound was 3:4:5-trichlorobenzyl alcohol followed by 4-chloro-3:5-dimethyl-, 3:4-dichloro- and 2:4-dichlorobenzyl alcohols, but saturated aqueous solutions of the last three compounds were more rapidly bactericidal. Pharmacological tests have shown that 2:4-dichlorobenzyl alcohol has low toxicity.

Antibacterial properties were first attributed to benzyl alcohol by Nördlinger[1] in 1915. A further study by Macht and Satani[2] showed that a 0·2 per cent aqueous solution was bacteriostatic to *Staphylococcus aureus* and *Escherichia coli* and a 3 per cent solution was lethal to these organisms in 10 minutes.

Benzyl alcohol also found some application in medicine for its local anaesthetic properties[3,4], and in recent years the alcohol has been used as a preservative. In 1952 Gershenfeld[5] indicated its value in parenteral solutions by showing that a 1 per cent aqueous solution was an effective bacteriostat against *Staph. aureus*, *E. coli*, *Bacillus subtilis*, *Bacillus mesentericus* and *Bacillus megatherium*. Further aspects on the use of benzyl alcohol as a preservative have been considered by Kleine, Millwood and Walther[6], and by Royce and Sykes[7].

Since halogenation of phenols produces compounds with enhanced antiseptic properties[8], we considered that chlorinated benzyl alcohols might possess improved antibacterial and antifungal activity. Preliminary experiments confirmed this and a wide range of substituted benzyl alcohols was prepared. These, together with other related, commercially available, compounds were tested against a variety of bacteria and fungi to evaluate their *in vitro* activities. Pharmacological toxicity tests were made on two of the more active compounds. When this work had been completed a war-time French patent[9] was found which attributed antiseptic properties to some substituted hydroxyalkyl- and hydroxyalkenylbenzenes.

Chemical

A series of substituted benzyl alcohols was prepared and experimental details are given for those compounds which have not previously been described in the literature.

2:4:5-*Trichlorobenzyl alcohol.* A mixture of 2:4:5-trichlorotoluene[10] (36·5 g., 0·186 mol.), sulphuryl chloride (25·25 g., 0·186 mol.), and

benzoyl peroxide (0·23 g.) (see Kharasch and Brown[11]) was heated under reflux for 3 hours and then distilled to give 2:4:5-trichlorobenzyl chloride (24·8 g., 58 per cent), b.p. 114 to 120°/4 to 5 mm. The chloride (30 g., 0·13 mol.) was stirred at 95° for 48 hours with a solution of potassium carbonate (13·8 g., 0·1 mol.) in water (100 ml.), the mixture cooled, and the product collected and crystallised from light petroleum (b.p. 60 to 80°) to give 2:4:5-*trichlorobenzyl alcohol* (11·9 g., 43 per cent), m.p. 111 to 113°. Found: C, 40·3; H, 2·3 per cent. $C_7H_5OCl_3$ requires C, 39·7; H, 2·4 per cent.

3:4:5-*Trichlorobenzyl alcohol.* Similar treatment of 3:4:5-trichlorotoluene[12] gave, via 3:4:5-trichlorobenzyl chloride, b.p. 121 to 124°/4·5 mm., 3:4:5-*trichlorobenzyl alcohol* which crystallised from light petroleum (b.p. 80 to 100°) as needles, m.p. 108 to 110°. Found: C, 39·7; H, 2·1 per cent.

2:3:*x*:*y*-*Tetrachlorobenzyl alcohol.* As described by Beilstein and Kuhlberg[13], benzyl chloride (233 g.) was chlorinated exhaustively in the presence of first iodine (1 g.) and then antimony trichloride (18 g.). Crystallisation from chloroform-methanol of the crude product gave 2:3:4:5:6-pentachlorobenzyl chloride (12 per cent), m.p. 98·5 to 100·5° (*idem*[13] give m.p. 103°). Using the method of Ross and Markarian[14] the chloride was converted into 2:3:4:5:6-pentachlorobenzyl alcohol, m.p. 194 to 196° (*idem*[14] give m.p. 197 to 198°). Distillation (15 cm. Hempel column) of the content of the above chloroform-methanol mother-liquor gave a fraction, b.p. 148 to 166°/4 mm., refractionated to give an oil, b.p. 120 to 130°/1·5 mm. Found: C, 32·0; H, 1·3 per cent. $C_7H_3Cl_5$ requires C, 31·8; H, 1·1 per cent. This oil (21·5 g.) was heated under reflux for 20 hours with anhydrous sodium acetate (26 g.) and glacial acetic acid (250 ml.), the mixture evaporated to 100 ml. and diluted with water (500 ml.). The oily acetate mixture (17·2 g.) was isolated with chloroform and then boiled under reflux for 1 hour with ethanolic sodium hydroxide (200 ml., 5 per cent). After evaporation to 100 ml., the mixture was diluted with water (300 ml.) and the gummy precipitate crystallised from chloroform to give the pentachlorobenzyl alcohol (1·75 g.). Repeated crystallisation from chloroform-light petroleum (b.p. 40 to 60°) of the content of the mother-liquor gave 2:3:*x*:*y*-*tetrachlorobenzyl alcohol* (0·5 g.) as needles, m.p. 128 to 130°. Found: C, 33·95; H, 1·8 per cent. $C_7H_4OCl_4$ requires C, 34·15; H, 1·6 per cent.

4-*Chloro*-3:5-*dimethylbenzyl alcohol.* 4-Chloro-3:5-dimethylbenzoic acid[15] (3·7 g., 0·02 mol.) was converted with boiling methanol (25 ml.) and concentrated sulphuric acid (0·68 ml.) (4 hours) into the methyl ester (3·8 g.) which was dissolved in dry ether (200 ml.) and added dropwise to a stirred mixture of lithium aluminium hydride (0·5 g., 0·013 mol.) in dry ether (150 ml.). After 2 hours the mixture was decomposed with dilute sulphuric acid and the ether-soluble product crystallised from light petroleum (b.p. 40 to 60°) to give the *alcohol* as needles (1·6 g., 47 per cent), m.p. 39 to 41°. Found: C, 63·6; H, 6·6 per cent. $C_9H_{11}OCl$ requires C, 63·4; H, 6·5 per cent.

4-*Iodo*-3:5-*dimethylbenzyl alcohol.* A mixture of 2-iodomesitylene[16] (78 g.) manganese dioxide[17] (27·8 g.), and 62 per cent sulphuric acid (96 ml.) was stirred at 65° for 8 hours. The cold mixture was diluted with water and the neutral product, isolated with ether, distilled to give 2-iodomesitylene (67 per cent recovery) and a semi-solid (6·9 g.), b.p. 120 to 160°/2 mm. The last was triturated with light petroleum (3 × 5 ml.) and the residue crystallised from methanol to give 2:4-diiodomesitylene as plates (1·1 g.), m.p. 83 to 84° (cf. Töhl and Eckel[18] who give m.p. 82 to 83°). Found: C, 29·4; H, 2·6; I, 67·7 per cent. Calc. for $C_9H_{10}I_2$: C, 29·0; H, 2·7; I, 68·3 per cent. Chromatography over alumina (100 g., Spence type "H") of the content of the light petroleum and methanol solutions gave a solid (3·1 g.) eluted with light petroleum (b.p. 40 to 60°) (500 ml.), which was crystallised first from methanol to give 2:4-diiodomesitylene (0·2 g.), and then from aqueous methanol to give 4-*iodo*-3:5-*dimethylbenzaldehyde* (1·85 g., 2 per cent) as needles, m.p. 65 to 67°. Found: C, 41·7; H, 3·6 per cent. $C_9H_{10}OI$ requires C, 41·5; H, 3·5 per cent. The 2:4-*dinitrophenylhydrazone* crystallised from chloroform as crimson needles, m.p. 283 to 284°. Found: N, 13·2 per cent. $C_{15}H_{13}O_4N_4I$ requires N, 12·7 per cent.

A solution of the aldehyde (3·7 g.) in methanol (20 ml.) was treated dropwise with a solution of potassium borohydride (0·2 g.) in water (5 ml.). After 2 hours, the mixture was evaporated under reduced pressure to 8 ml., cooled, acidified, diluted with water, and the product isolated with ether. Crystallisation from light petroleum (b.p. 40 to 60°) gave 4-*iodo*-3:5-*dimethylbenzyl alcohol* (2·2 g., 60 per cent) as elongated prisms, m.p. 82 to 83°. Found: C, 41·1; H, 4·2 per cent. $C_9H_{11}OI$ requires C, 41·2; H, 4·2 per cent.

6-*Nitropiperonyl alcohol.* A suspension of 6-nitropiperonal[19] (20 g., 0·1 mol.) in methanol (250 ml.) was stirred during the dropwise addition of potassium borohydride (2 g., 0·038 mol.) in water (20 ml.). After 1 hour, the solution was acidified and poured into water. Crystallisation of the precipitate from benzene gave the *alcohol* (17·4 g., 86 per cent) as pale yellow needles, m.p. 122 to 123°. Found: C, 48·5; H, 3·5; N, 7·3 per cent. $C_8H_7O_5N$ requires C, 48·7; H, 3·55; N, 7·1 per cent. Mono acetyl derivative: m.p. 149 to 150°. Found: C, 50·5; H, 3·7; N, 6·2 per cent. $C_{10}H_9O_6N$ requires C, 50·2; H, 3·8; N, 5·9 per cent.

2:4-*Dichlorobenzyl propionate**. The *ester*, b.p. 114°/1 mm., was prepared by treatment of 2:4-dichlorobenzyl alcohol (7·2 g.) with boiling propionic anhydride (9·5 ml.) and a trace of concentrated sulphuric acid for 4 hours. Found: C, 51·4; H, 4·4 per cent. $C_{10}H_{10}O_2Cl_2$ requires C, 51·5; H, 4·3 per cent.

2:4-*Dichlorobenzyl methyl ether.* A mixture of 2:4-dichlorobenzyl chloride (31·5 g., 0·161 mol.) and sodium methoxide (from 3·8 g., 0·165 g. atom sodium) in methanol (250 ml.) was heated under reflux for 6 hours, cooled, filtered, and evaporated. Partition of the residue between water

* Prepared by Mr. J. Fraser.

and ether and distillation of the content of the ether phase, gave 2:4-*dichlorobenzyl methyl ether* (18 g., 59 per cent), b.p. 73°/0·6 mm. Found: C, 50·2; H, 4·3 per cent. $C_8H_8OCl_2$ requires C, 50·3; H, 4·4 per cent.

Sodium benzyl sulphate. A solution of benzyl alcohol (5 g., 0·046 mol.) in dry pyridine (25 ml.) was treated with sulphamic acid (13·5 g., 0·138 mol.) and the mixture heated to 83°. When the exothermic reaction had abated, heating on the steam bath was continued for 15 minutes. The cold mixture was filtered, the filtrate evaporated under reduced pressure, and the residue treated with aqueous *N*-sodium hydroxide (46·3 ml., 1 equiv.). On evaporation, the salt separated; crystallisation from a little water gave *sodium benzyl sulphate* (2 g., 20 per cent) as plates, m.p. 208 to 210°. Found: C, 39·6; H, 3·3 per cent. $C_7H_7O_4SNa$ requires C, 40·0; H, 3·3 per cent.

Sodium 2:3:4:5:6-*pentachlorobenzyl sulphate monohydrate* was prepared in a similar way and crystallised from very dilute (pH 8) sodium hydroxide as elongated plates (41 per cent), m.p. 270 to 272° (slow rate of heating). Found, in a sample dried over phosphorus pentoxide in a vacuum at 22°: C, 21·6; H, 1·1; H_2O 4·7 per cent. $C_7H_2O_4SCl_5Na$, H_2O requires C, 21·0; H, 1·0; H_2O, 4·5 per cent.

Some of the remaining alcohols were obtained commercially; the others were prepared by known methods and their properties agreed with those cited in the literature.

Microbiological Evaluation

Inhibition tests were made on serial dilutions of all compounds at pH 4·5, 6·5, and 8·0 and lethal tests on unbuffered solutions of those compounds which showed good inhibitory activity. Where necessary the compound was first dissolved in alcohol such that the final concentration of alcohol did not exceed 2 per cent.

The inhibition tests were made by dissolving the test compound in 10 ml. amounts of a tryptic digest glucose broth diluted ten times with tap water and buffered to the requisite pH with a sodium phosphate, citric acid buffer. The organisms used were Gram-positive bacteria (G+B), Gram-negative bacteria (G−B) and mixed mould spores, each group being considered separately. For the tests with bacteria, 24 hour cultures in nutrient broth were used, equal volumes of each strain being mixed together just before use; the G+B comprised *Staph. aureus* (3 strains), *Staph. albus* and *Streptococcus faecalis* and the G−B were *Proteus vulgaris*, *E. coli* and several *Pseudomonas* cultures including ten strains of *Pseudomonas pyocyanea*. For the tests with mould spores, seven-day old cultures of species of *Aspergillus*, *Penicillium*, *Cladosporium* and *Mucor* grown on honey agar were gently scraped off and suspended in sterile water containing a small amount of wetting agent (1 in 1000 Solution of Sulphestol). After mixing and straining to remove any large clumps, this suspension, containing approximately 1×10^8 spores per ml., was used.

To each 10 ml. of the prepared dilution of the test compound, 0·05 ml. of the suspension of the appropriate organisms was added and after incubation for 5 days at 25° the results were noted. The minimum

inhibitory dilution was taken as that dilution which just prevented growth.

TABLE I

ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF NUCLEAR-SUBSTITUTED BENZYL ALCOHOLS

Compound	Substituent group(s) introduced CH_2OH (2)	(3)	(4)	(5)	(6)	Approx. solubility in water at 20° (1 in −)	Minimum inhibitory concentration at pH 6·5 (1 in −) G + B	G − B	Moulds
Benzyl alcohol	—	—	—	—	—	25	300	200	300
4-Chlorobenzyl alcohol	—	—	Cl	—	—	400	400	400	800
2:4-Dichlorobenzyl alcohol	Cl	—	Cl	—	—	1000	2000	4000	6600
3:4-Dichlorobenzyl alcohol	—	Cl	Cl	—	—	1250	4000	4000	6600
2:4:5-Trichlorobenzyl alcohol	Cl	—	Cl	Cl	—	6000	10,000	N.A.M.S.	10,000
3:4:5-Trichlorobenzyl alcohol	—	Cl	Cl	Cl	—	6000	20,000	10,000	20,000
2:3:x:y-Tetrachlorobenzyl alcohol	Cl	Cl	←	— 2Cl —	→	15,000	20,000	N.A.M.S.	N.A.M.S.
Pentachlorobenzyl alcohol	Cl	Cl	Cl	Cl	Cl	500,000	N.A.M.S.	N.A.M.S.	N.A.M.S.
2-Bromobenzyl alcohol	Br	—	—	—	—	750	750	750	750
4-Bromobenzyl alcohol	—	—	Br	—	—	450	2000	1000	1000
4-Iodobenzyl alcohol	—	—	I	—	—	2400	2400	N.A.M.S.	2400
4-Methylbenzyl alcohol	—	—	CH_3	—	—	160	500	500	500
2:4-Dimethylbenzyl alcohol	CH_3	—	CH_3	—	—	220	1000	1000	1000
4-Chloro-3:5-dimethylbenzyl alcohol	—	CH_3	Cl	CH_3	—	1800	6600	4000	6600
4-Iodo-3:5-dimethylbenzyl alcohol	—	CH_3	I	CH_3	—	10,000	20,000	N.A.M.S.	10,000
2-Hydroxybenzyl alcohol	OH	—	—	—	—	15	500	200	200
3-Hydroxybenzyl alcohol	—	OH	—	—	—	15	200	200	200
4-Hydroxybenzyl alcohol	—	—	OH	—	—	150	200	200	200
Piperonyl alcohol	—	O, O, CH_2		—	—	350	N.A.M.S.	N.A.M.S.	N.A.M.S.
6-Nitropiperonyl alcohol	—	O, O, CH_2		—	NO_2	6000	6000	N.A.M.S.	N.A.M.S.
Vanillyl alcohol	—	OCH_3	OH	—	—	500	N.A.M.S.	N.A.M.S.	N.A.M.S.
Anisic alcohol	—	—	OCH_3	—	—	500	500	N.A.M.S.	N.A.M.S.
2-Nitrobenzyl alcohol	NO_2	—	—	—	—	200	2000	1000	2000
3-Nitrobenzyl alcohol	—	NO_2	—	—	—	1700	N.A.M.S.	N.A.M.S.	N.A.M.S.
4-Nitrobenzyl alcohol	—	—	NO_2	—	—	500	660	660	660
4-Cyanobenzyl alcohol	—	—	CN	—	—	30	350	350	350
4-Hydroxycarbonylbenzyl alcohol	—	—	CO_2H	—	—	100	500	350	100
2:4-Dimethyl-5-hydroxymethylbenzyl alcohol	CH_3	—	CH_3	CH_2OH	—	820	N.A.M.S.	N.A.M.S.	N.A.M.S.
Cumic alcohol	—	—	C_3H_7	—	—	8000	N.A.M.S.	N.A.M.S.	N.A.M.S.

N.A.M.S. = Not active at maximum solubility.

For the lethal tests, in which bacteria only were used, dilutions of the selected compounds were prepared in water and 0·1 ml. of a combined mixture of organisms, as used in the inhibition test, was added to 10 ml. of each dilution. After 15 minutes, and at intervals during 24 hours, 0·1 ml. amounts were transferred to tubes each containing 10 ml. of saline and 1 ml. from each tube was plated with nutrient agar. Counts of the surviving organisms were made after incubation at 37°. The lethal time for a particular concentration was taken as being the time at which no surviving organisms were found. This was equivalent to a kill of over 99·99 per cent.

Results

The results of the inhibitory tests and the approximate solubilities of the compounds in water at 20° are given in Tables I and II. The majority of compounds showed little difference in activity over the pH range tested and consequently results at pH 6·5 only are shown.

The results of the lethal tests are given in Table III.

Discussion of Microbiological Results

Table I summarises the bacterial and fungal inhibitory properties of the nuclear-substituted benzyl alcohols. Introduction of a hydroxyl, methylenedioxy, cyano, or carboxyl group caused little or no increase in activity, whilst an alkoxyl or hydroxymethyl group removed activity. Nitration had the greatest effect at the 2-position whilst the 3-compound was inactive.

The most effective compounds were the halogenated benzyl alcohols. As the number of chlorine substituents increased the activity rose until a maximum was reached with 3 : 4 : 5-trichlorobenzyl alcohol. A tetrachloro alcohol was active only against Gram-positive bacteria and the fully substituted alcohol was inactive. The decrease in activity of the last two compounds was attributed to their very low solubility in water. 4-Bromobenzyl alcohol was more effective than the 2-isomer and was also more active than the 4-chloro compound whilst 4-iodobenzyl alcohol was much less soluble and inactive against Gram-negative bacteria. A methyl substituent in the nucleus gave an alcohol of increased activity but a larger grouping such as *iso*propyl decreased the solubility and activity was lost. Berger, Hubbard and Ludwig[20] found that the most active members of a series of phenyl ethers of glycerol, propylene and trimethylene glycols possessed a 4-chloro-3 : 5-dimethyl substitution pattern (cf. chloroxylenol), but in the benzyl alcohol series the activity of the 4-chloro-3 : 5-dimethyl compound was only of the same order as that of the two dichloro-alcohols examined. The 4-iodo-3 : 5-dimethyl- compound was much less soluble and was inactive against Gram-negative bacteria.

The effect of side-chain substitution is summarised in Table II. Activity was destroyed by esterification, etherification or sulphation of the hydroxyl-group. Substitution at the carbon atom by methyl, carboxyl, alkoxycarbonyl, or hydroxymethyl groups produced no increase in activity.

SOME SUBSTITUTED BENZYL ALCOHOLS

One phenyl substituent produced marked inhibition against Gram-positive bacteria and moulds but the compound lacked effect on Gram-negative bacteria, whilst triphenyl carbinol was so insoluble as to be devoid of activity. The more soluble hydrochlorides of diphenyl (piperid-2-yl-)- and diphenyl (piperid-4-yl) methanols had low activity.

TABLE II

ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF SIDE-CHAIN SUBSTITUTED BENZYL ALCOHOLS

Compound	Substituent group(s) introduced (H)	(OH)	(H)	Approx. solubility in water at 20° (1 in −)	Minimum inhibitory concentration at pH 6·5 (1 in −) G + B	G − B	Moulds
Benzyl alcohol	—	—	—	25	300	200	300
Benzyl methyl ether	—	OCH_3	—	330	N.A.M.S.	N.A.M.S.	N.A.M.S.
Sodium benzyl sulphate	—	$NaSO_4$	—	6	<100	<100	<100
Sodium pentachlorobenzyl sulphate	—	$NaSO_4$	—	850	N.A.M.S.	N.A.M.S.	N.A.M.S.
2:4-Dichlorobenzyl propionate	—	$CH_3CH_2CO_2$	—	5000	N.A.M.S.	N.A.M.S.	N.A.M.S.
2:4-Dichlorobenzyl methyl ether	—	OCH_3	—	6000	N.A.M.S.	N.A.M.S.	N.A.M.S.
Phenylethylene glycol	CH_2OH	—	—	3·5	<100	<100	<100
α-Phenylethanol	CH_3	—	—	150	300	300	300
Diphenyl carbinol	C_6H_5	—	—	2000	2000	N.A.M.S.	2000
Triphenyl carbinol	C_6H_5	—	C_6H_5	100,000	N.A.M.S.	N.A.M.S.	N.A.M.S.
Mandelic acid	CO_2H	—	—	6	500	100	<100
Ethyl mandelate	$CO_2C_2H_5$	—	—	100	500	200	200
Benzilic acid	CO_2H	—	C_6H_5	250	500	250	N.A.M.S.
α-Phenyl*iso*propanol	CH_3	—	CH_3	200	200	200	200
Diphenyl(piperid-2-yl)methanol hydrochloride	C_6H_5	—	$C_5NH_{10}HCl$	20	100	200	100
Diphenyl(piperid-4-yl)methanol hydrochloride	C_6H_5	—	$C_5NH_{10}HCl$	80	350	350	200

N.A.M.S. = Not active at maximum solubility.

The results of the lethal tests with some of the more inhibitory compounds are summarised in Table III, and show that 2:4-dichloro-, 3:4-dichloro-, 4-chloro-3:5-dimethylbenzyl alcohols have pronounced lethal properties. 3:4:5-Trichlorobenzyl alcohol, which showed maximum activity in the inhibition test, was lethal in 24 hours only as a saturated solution.

PHARMACOLOGICAL STUDY OF SELECTED COMPOUNDS

Approximate lethal doses (LD50) in mice were determined on selected compounds and the results are shown in Table IV. The apparent decrease in toxicity of 2:4-dichlorobenzyl alcohol on subcutaneous injection is under investigation.

A more detailed toxicological study was then carried out on 2:4-dichlorobenzyl alcohol as it appeared to be the least toxic of the dichloro-compounds.

TABLE III

LETHAL ACTIVITY OF SELECTED COMPOUNDS

Compound	Concentration (1 in —)	*Lethal time
Benzyl alcohol	50	1 hour
4-Chlorobenzyl alcohol	400	24 hours
2:4-Dichlorobenzyl alcohol	1000	1 hour
3:4-Dichlorobenzyl alcohol	1250	1 hour
3:4:5-Trichlorobenzyl alcohol	6000	24 hours
4-Bromobenzyl alcohol	450	24 hours
4-Methylbenzyl alcohol	200	24 hours
4-Chloro-3:5-dimethylbenzyl alcohol	1800	1 hour

* Time to reduce viable count to 100 per ml.

TABLE IV

APPROXIMATE LETHAL DOSES (LD 50) OF SELECTED COMPOUNDS TO MICE

Compound	Vehicle	Acute oral toxicity (mg./kg.)	Acute subcutaneous toxicity (mg./kg.)
Benzyl alcohol	Aqueous solution	1150	950
2:4-Dichlorobenzyl alcohol	Acacia suspension	1300	1770
3:4-Dichlorobenzyl alcohol	Acacia suspension	620	700

Short-term chronic toxicity. Three groups of newly-weaned albino rats, with five males and five females in each, were given daily oral doses in propylene glycol of 50, 150 or 500 mg./kg., 6 days each week for 3 weeks. An additional group was given propylene glycol alone as a control. The rats were weighed daily and those receiving the largest dose were examined for haematological effects during the last week of dosing. At autopsy the livers and kidneys were weighed and specimens of the major organs examined histologically.

Increases in weight were the same in all groups, and there were no pathological effects on blood or organs.

Local toxicity to rabbit eye. Two drops of a 0·08 per cent aqueous solution were instilled into the cupped eyelid of one eye of each rabbit, and maintained there for 60 seconds before the surplus solution was allowed to drain away; the other eye of each rabbit was treated with water as a control. Applications were made on 4 successive days to three rabbits without having any irritant effects.

Single applications of 1 and 5 per cent solutions in polyethylene glycol 400 and of a 1·5 per cent solution in propylene glycol had some irritant effects which were no greater than those produced by the solvents alone.

Skin sensitisation. Two methods were used to test for skin sensitisation in the guinea pig. In the first method, ten applications of a 2 per cent

solution in acetone were made to the shaved skin of three guinea pigs over a period of 3 weeks. The first dose was 0·05 ml. and the nine subsequent doses were 0·1 ml. each. Two weeks after the last sensitising dose the challenging dose of 0·05 ml. was given and the skin examined 24 hours later. There were no reactions from either the first sensitising or the challenging dose.

In the second method the dosage regime was similar except that a 0·1 per cent aqueous solution was given intradermally. The reaction produced in the sensitised guinea pigs was slight and no more than that produced by the first sensitising dose.

From this study it appears that 2:4-dichlorobenzyl alcohol has a low toxicity and shows good activity against a wide range of bacteria and moulds.

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DISCUSSION

The Paper was presented by MR. A. H. FENTON.

THE CHAIRMAN. Was any information available about the absorption of 2:4-dichlorobenzyl alcohol by rubber caps?

DR. K. R. CAPPER (London). The reference to the partition of 2:4-dichlorobenzyl alcohol in rubber in MR. SYKE'S Paper indicated that the concentration might fall below the minimum inhibitory figure for Gram-positive bacteria. Do any other compounds show greater promise?

DR. F. HARTLEY (London). Was any information available about other properties of the compounds and of the stability of 2:4-dichlorobenzyl alcohol? If the halogen group was easily removed, incompatibilities might arise. In the case of emulsions it was important to know what chemical changes occurred if a substance was transferred from one phase to another.

DISCUSSION

PROFESSOR G. BROWNLEE (London). In Table IV the acute oral toxicity of an aqueous solution of benzyl alcohol was compared with that of an acacia suspension and until the range of the approximate lethal doses, was known it was difficult to say whether there was any difference between the first two compounds. Benzyl alcohol was more toxic in the subcutaneous tests, suggesting that it was poorly absorbed from the gastro-intestinal tract. What was the absorption from under the skin? It would be useful to have the figures for the intravenous toxicity.

MR. G. SYKES (Nottingham). Absorption into rubber was not connected with chemical activity.

DR. J. C. PARKINSON (Brighton). Was 2:4-dichlorobenzyl alcohol stable when autoclaved?

MR. H. G. ROLFE (London). For what purpose was the substance intended to be used, as an ingredient of throat lozenges or of injections?

DR. H. S. BEAN (London). How active were the compounds in fairly high dilution? In addition to the lethal concentration it was necessary to know the concentration exponent.

DR. A. H. BECKETT (London). It seemed from the results that Ferguson's Law was applying as the activity was proportional to the degree of saturation in the biophase. Had any work been done on these compounds in the presence of non-ionic compounds which would easily inactivate phenols?

DR. A. M. COOK (London). Why had mixtures of different strains and species been used? As 2:4-dichlorobenzyl alcohol might be used in throat lozenges was there any further information on the spectrum of this compound particularly on diphtheroids and Gram-negative cocci? An organism resistant to a general bactericide might not be resistant to a chlorinated compound since there was a great deal of specificity with increasing chlorination.

DR. LESSEL replied. The number of animals and dose levels were insufficient to show whether there was a statistically significant difference between the acute subcutaneous and acute oral figures. It was difficult to compare results of aqueous solutions and of acacia suspensions. He agreed that the figures suggested that there was not a vast difference between benzyl alcohol and the 2:4-derivative. Work was in progress to establish the reason for the lower subcutaneous toxicities. Intravenous injections had not been used.

MR. FENTON replied. 2:4-Dichlorobenzyl alcohol was readily absorbed by rubber; the other compounds were not investigated in this connection. The compound would pass into the oil phase of an emulsion. In the neutral range it appeared to be stable in water. A 0·1 per cent aqueous solution at room temperature or at 37° for two months showed little change. He had no specific information about the stability of an aqueous solution on autoclaving. The material had been formulated in a throat lozenge but it might have application as a preservative or as a therapeutic

agent. It seemed that both anionic and non-ionic surface-active agents inactivated these compounds to some extent.

MR. CARTER replied. Mixtures of organisms had been used as a matter of expediency. The compound had been effective against a number of organisms common in the mouth and throat but it had not been tested against Gram-negative cocci, which do not cause infection in the throat.