THE BIOLOGICAL ACTIVITY OF CERTAIN DERIVATIVES OF DDT

by

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THE BIOLOGICAL ACTIVITY OF CERTAIN DERIVATIVES OF DDT

INTRODUCTION

It has long been recognized that the discovery of a law relating biological activity of organic compounds to their structures would be of benefit to the investigator who is unceasingly searching for agents exhibiting such activity. Selectively toxic agents in particular are needed, for agricultural foodstuffs must be protected from weeds and pests of many kinds, and economic animals are troubled with biological enemies of all types. Man himself is not respected by these parasites.

Up to this period of development in various fields of the sciences, attempts have been made to correlate chemical structure with specific selective toxic activity. The insecticide DDT, 1,1-bis-(p-chlorophenyl)2,2,2-trichloroethane, may be taken as a prime example, for because of its great economic importance many investigators have given their time to considering this biologically active compound.

A first group considers the toxic portion of the DDT molecule as residing in the linked p-chlorobenzene rings, while the -CCl₃ grouping is believed to impart lipid solubility (22, pp.892-928). Another group suggests
that the linked p-chlorobenzene rings impart lipoid solubility, while toxicity is due rather to the ability to split out hydrogen chloride from the remainder of the molecule (25, pp.512-513). A third group casts doubt on either of these speculations and considers that perhaps too simple a relationship is sought for such a complex phenomenon (6, pp.169-170).

A more theoretical approach has been taken recently by yet another group who feels there is little basis for assigning more or less independent lipoid solubility and toxic properties to parts of the DDT molecule (38, pp.2990-2999). Rather they consider that toxicity is due to the molecule as a whole and is explainable by means of a "tri-hedralized" structure resulting from interaction of the p-substituted benzene rings and the -CCl₃ group or groupings of similar and greater size.

However, a law relating toxicity to structure broad enough to systematize and simplify the development of successful agents needed to combat parasites afflicting Man and his food sources has not been forthcoming.

The first step in any logical approach as to how biologically active substances work is to record the various biological, physical and chemical properties of active compounds. Until enough relevant facts are collected, progress toward such an end is slow and time
consuming. Therefore, it is hoped that the data given herein will add a small part to the necessary accumulation of evidence that may eventually settle the question of this correlation or lack of correlation between chemical structure and toxicity.

The diphenylmethylene radical, \( \text{Q} - \text{C} - \text{Q} \), was chosen for this study of structural relationship to toxicity. A review of the literature shows that this molecular arrangement is contained in a number of biologically active compounds.

A preliminary investigation was first made in which several derivatives of the basic structure were synthesized in order to ascertain if certain functional groups would most readily lend themselves to study. The results of this preliminary investigation indicated that additional time might profitably be spent contrasting the biological activity of ketone derivatives with the corresponding alcohols.

Accordingly, there were synthesized a series of ketones, secondary alcohols and in the cases found feasible, the tertiary alcohols. Chemical and biological data on these compounds are given herein. Other derivatives studied in the preliminary investigation are also listed in an addendum.

The compounds were screened on mosquito larvae and six microorganisms chosen for this purpose.
Certain of the compounds under discussion in this study have been examined from the viewpoint of biological activity by other workers. Following is given a brief summary of the findings relevant to this investigation.

Benzophenone has been extensively investigated as a possible insecticide (33, pp.985-991) and 24, pp.43-47). It is also known to be somewhat bacteriostatic against tubercle bacilli (15, pp.153-156).

4,4'-Dichlorobenzophenone is known to be bacteriostatic against tubercle bacilli (15, p.154) and in addition shows some insecticidal properties (27, p.877).

Phenyl 2-thienyl ketone shows some toxic properties toward the body louse egg (11, p.33).

4,4'-Bis-(dimethylamino)benzophenone shows some slight bacteriostatic effect on tubercle bacilli (15, p.153-156).

Benzhydrol has been extensively investigated for insecticidal properties and shows some activity (36, p.64) and 10, p.477).

4,4'-Dichlorobenzhydrol has aroused some interest as an insecticide (27, p.877) and 36, p.64).

4,4'-Dimethoxybenzhydrol (36, p.64) shows some insecticidal activity against cockroaches and is known to be bacteriostatic toward tubercle bacilli (15, p.153-156).
1,1-Bis-(p-chlorophenyl)ethanol has been demonstrated an active insecticide (16, pp.361-362) and B, pp.168-171). It inhibits the growth of Cryptococcus neoformans in vitro (40, p.20).

Bis-(p-chlorophenyl)methane is somewhat toxic toward mosquito larvae (9, pp.118-119), insects (27, p.877) and rabbits (23, pp.127-142).

Some insecticidal activity has been demonstrated for 1,1-bis(p-chlorophenyl)ethylene (27, p.877).

1,1-Bis-(p-chlorophenyl)ethane shows toxic properties against insects (44, p.207) and 27, p.877).

In summary, then, chemical and biological data are presented herein contrasting ketone and alcohol derivatives of the diphenylmethylen radical.
EXPERIMENTAL

Benzophenone

Benzophenone was obtained from the Eastman Kodak Company, Organic Sales Division, Rochester 4, New York. Its melting point was found to be 47-48 degrees Centigrade and was used with no further purification.

4,4'-Dichlorobenzophenone

4,4'-Dichlorobenzophenone was prepared according to the directions of Newton and Groggins (34, p.1398). Yields of 82.07 per cent melting at 148 degrees Centigrade were reported. A yield of 78 per cent was obtained melting at 148 degrees Centigrade.

4,4'-Dimethoxybenzophenone

4,4'-Dimethoxybenzophenone was obtained from the Eastman Kodak Company, Organic Sales Division, Rochester 4, New York. Its melting point was found to be 143.5-145 degrees Centigrade and was used without further purification.

4,4'-Dichloro-3,3'-dinitrobenzophenone

4,4'-Dichloro-3,3'-dinitrobenzophenone was prepared according to the directions of Backeberg and Marais (1, p.305). A melting point of 131.5 degrees Centigrade was
reported. A yield of 74.6 per cent was found melting at 132.0 degrees Centigrade.

4,4'-Dichloro-3,3'-diaminobenzophenone

4,4'-Dichloro-3,3'-diaminobenzophenone was prepared according to the directions of Montagne (32, pp.1030-1031). A melting point of 167.5 degrees Centigrade was reported. A yield of 77.0 per cent was found melting at 167.5 degrees Centigrade.

4-Chlorophenyl 2-thienyl ketone

4-Chlorophenyl 2-thienyl ketone was prepared according to the directions of Ng, Ph. Buu-Hoi, et al. (7, p.1095). A yield of 70.0 per cent melting at 99 degrees Centigrade was reported. A yield of 71.5 per cent melting at 99 degrees Centigrade was found.

Phenyl 2-thienyl ketone

Phenyl 2-thienyl ketone was prepared according to directions given in Organic Syntheses (4, pp.520-521). A yield of 90 per cent melting at 55-56 degrees Centigrade was reported. Ninety per cent yield melting at 55-56 degrees Centigrade was found.
4,4'-Bis-(dimethylamino)benzophenone

4,4'-Bis-(dimethylamino)benzophenone was obtained from Eastman Kodak Company, Organic Sales Division, Rochester 4, New York. Its melting point was found to be 173 degrees Centigrade and was used with no further purification.

4-Fluoro-3-methylbenzophenone

4-Fluoro-3-methylbenzophenone was prepared as follows: In a 250 milliliter flask fitted with stirrer, dropping funnel and reflux condenser were placed 20.0 grams o-fluorotoluene in 150 milliliters dry carbon disulfide. To this were added 27.0 grams anhydrous aluminum chloride. Then 25.6 grams benzoyl chloride were slowly introduced by means of the dropping funnel and the mixture finally refluxed three hours. A condenser was attached to one of the necks and the solvent removed. The residue was poured on 100 grams of ice to which had been added 30 milliliters concentrated hydrochloric acid. The mixture was extracted with 100 milliliters of ether, washed once with 50 milliliters of water, once with 50 milliliters dilute sodium hydroxide and twice more with water. The solvent was removed and the colorless 4-fluoro-3-methylbenzophenone distilled at 176 degrees Centigrade under a pressure of ten millimeters of mercury. The liquid soon solidified and was twice crystallized from Skelly-solve F to give 25.1 grams (64.5 per cent
yield) of the 4-fluoro-3-methylbenzophenone melting at 54 degrees Centigrade. The calculated percentages of carbon and hydrogen are 78.49 and 5.19; found 78.48 and 5.18. This compound is identical in respect to melting point and mixed melting point to the 4-fluoro-3-methylbenzophenone given below prepared by oxidation of 1-(4-fluoro-3-methylphenyl) 1-phenylethylene obtained in turn from the elucidated structure of 4-fluoro-3-methylacetophenone and phenylmagnesium bromide. The 2,4-dinitrophenylhydrazone melts at 188-189 degrees Centigrade. Calculated percentages for carbon and hydrogen are 60.91 and 3.83; found 60.97 and 3.76.

**Benzhydrol**

Benzhydrol was prepared according to directions given in Organic Syntheses (3, pp.90-91). A yield of 69-72 per cent was reported melting at 68 degrees Centigrade. A yield of 70 per cent melting at 68 degrees Centigrade was found.

**4,4′-Dichlorobenzhydrol**

4,4′-Dichlorobenzhydrol was prepared according to directions given by Montagne (30, pp.115-116). A melting point of 94 degrees Centigrade was reported. A yield of 85 per cent melting at 94 degrees Centigrade was found.
4,4'-Dimethoxybenzhydrol

4,4'-Dimethoxybenzhydrol was prepared according to the method of Schnackenberg, et al. (41, p.655). A product melting at 72 degrees Centigrade was reported. A yield of 71 per cent melting at 72 degrees Centigrade was obtained.

4,4'-Dichloro-3,3'-dinitrobenzhydrol

4,4'-Dichloro-3,3'-dinitrobenzhydrol was prepared in the following manner: In a 500 milliliter round-bottom flask was placed a solution of 35.9 grams of pure aluminum isopropoxide in 250 milliliters dry isopropyl alcohol and 20.0 grams of 4,4'-dichloro-3,3'-dinitrobenzophenone. A short reflux condenser was attached to the flask, but no water run through the cooling jacket. To the top of the condenser was attached a water-cooled condenser set for distillation. A boiling chip was added and the solution refluxed at such a rate that five to ten drops of distillate were collected per minute. After two hours when no more acetone was found to be present in the distillate, water was run through the upright condenser keeping the solution under total reflux for fifteen minutes. On retesting the distillate with 0.1 per cent solution of 2,4-dinitrophenyl-hydrazine for acetone and obtaining a negative test, the excess isopropyl alcohol was removed under slightly
diminished pressure. The cooled mass was hydrolyzed with 35 milliliters of concentrated hydrochloric acid and 200 milliliters water. An oil separated which was extracted with ether and washed with 100 milliliters water in two portions. On removal of the solvent and after standing over night, the residue solidified, was crystallized twice from benzene and thoroughly dried to give 18.2 grams of slightly yellow 4,4'-dichloro-3,3'-dinitrobenzhydrol melting at 110-111 degrees Centigrade. Calculated percentages for carbon and hydrogen are 45.50 and 2.35; found 45.62 and 2.39. No precipitate was obtained with 2,4-dinitrophenylhydrazine under conditions that gave a precipitate with the original ketone.

4,4'-Dichloro-3,3'-diaminobenzhydrol

4,4'-Dichloro-3,3'-diaminobenzhydrol was prepared as follows: Two grams of 4,4'-dichloro-3,3'-diaminobenzophenone were dissolved in 150 milliliters of anhydrous methanol in a flask fitted with a calcium chloride tube. Five tenths grams sodium borohydride were added and the mixture was allowed to stand over night at room temperature. The solution was then made strongly acidic with dilute hydrochloric acid, then strongly basic with dilute sodium hydroxide. Crystals separated when the solution was poured
into twice its volume of water and were filtered and dried. One recrystallization from a benzene Skelly-solve F mixture gave 1.81 grams (90 per cent yield) of slightly yellow crystals of 4,4'-dichloro-3,3'-diaminobenzhydrol melting at 118.5 degrees Centigrade as reported by Montagne (31, pp.2260-2261) for this compound.

4-Chlorophenyl-2-thienylmethanol

4-Chlorophenyl-2-thienylmethanol was prepared as follows: In a 100 milliliter round-bottom flask fitted with a reflux condenser were placed five grams of 4-chlorophenyl 2-thienyl ketone, 60 milliliters 95 per cent ethanol, 10 milliliters concentrated ammonium hydroxide and ten grams of aluminum amalgam. The mixture was refluxed six hours and filtered while hot. After cooling, the filtrate was poured into twice its volume of water. After standing 48 hours, crystals separated. On filtering, drying and recrystallizing from Skelly-solve B, 3.9 grams were obtained (77.5 per cent yield) melting at 59-60 degrees Centigrade as reported by Hamlin, et al. for this compound (19, p.2732).

Phenyl-2-thienylmethanol

Phenyl-2-thienylmethanol was prepared by following the procedure of Minnis (28, p.2144). A melting point of
57-58 degrees Centigrade was reported. A yield of 79 per cent was obtained melting at 57-58 degrees Centigrade.

4,4'-Bis-(dimethylamino)benzhydrol

4,4'-Bis-(dimethylamino)benzhydrol was prepared in the manner described by Mastagli (26, p.1657). 312 grams potassium metal were dissolved in ten milliliters dry benzyl alcohol. Two grams of 4,4'-Bis-(dimethylamino)benzophenone were added and the mixture was heated just below reflux for two hours. Water was added and the solvent steam distilled. The residue was poured into 500 milliliters of water and after standing over night the crystals were filtered and dried. Crystallization from Skelly-solve B gave 1.81 grams (90 per cent) of the hydrol melting at 103 degrees Centigrade as reported by Mohlañ, et al. (29, p.360). Mastagli reported a 96 degrees Centigrade melting point.

4-Fluoro-3-methylbenzhydrol

4-Fluoro-3-methylbenzhydrol was prepared as follows: In a 100 milliliter round-bottom flask fitted with a mechanical stirrer were placed eight grams sodium hydroxide, eight grams of 4-fluoro-3-methylbenzophenone, 50 milliliters of 95 per cent ethanol and eight grams zinc powder. The mixture was refluxed two hours, after which it was
filtered while still hot. The residue was washed twice with 20 milliliter portions of hot alcohol. The filtrate was neutralized with concentrated hydrochloric acid, extracted with 50 milliliters of ether and the ether layer washed twice with 20 milliliter portions of water. The solvent was removed and the hydrol distilled at 191 degrees Centigrade under a pressure of 14 millimeters of mercury to give 7.1 grams of colorless 4-fluoro-3-methylbenzhydrol (88 percent yield). The percentages of carbon and hydrogen were calculated to be 77.76 and 6.06. The percentages were found to be 78.04 and 6.03. A sample of the liquid gave no precipitate with 2,4-dinitrophenylhydrazine under conditions that a precipitate was obtained from the 4-fluoro-3-methylbenzophenone.

α-Methylbenzhydrol

α-Methylbenzhydrol was prepared according to directions found in Organic Syntheses (3, pp.226-227). Yields up to 75 per cent were reported. A yield of 69 per cent was obtained after recrystallization from Skelly-solve F melting at 81 degrees Centigrade as reported by Klages (21, p.2646).

1,1-Bis-(p-chlorophenyl)ethanol

1,1-Bis-(p-chlorophenyl)ethanol was prepared according to directions given by Grummitt, et al. (17, p.2265).
A yield of 89 per cent was reported melting at 67-68 degrees Centigrade. An 85 per cent yield was obtained melting at 67-68 degrees Centigrade.

1,1-Bis-(p-methoxyphenyl)ethanol

1,1-Bis-(p-methoxyphenyl)ethanol was prepared according to the method given by Grummitt, et al. (18, pp.1289-1920). A yield of 83 per cent was reported melting at 82-83.5 degrees Centigrade. A yield of 76 per cent was obtained melting at 83 degrees Centigrade.

1-Phenyl-1(2-thienyl)ethanol

1-Phenyl-1(2-thienyl)ethanol was prepared as follows: In a 500 milliliter three-neck flask were placed 648 grams of magnesium in 300 milliliters dry ether. To this was added slowly with stirring a total of 3.80 grams of methyl iodide in 50 milliliters dry ether. After the magnesium had disappeared, a total of five grams of phenyl-2-thienyl ketone in 50 milliliters dry ether was added with cooling. The mixture was refluxed one hour and poured on 50 grams of ice and 50 milliliters of water to which had been added five grams ammonium chloride. The ether layer was separated and washed twice with 25 milliliter portions of water. On removal of the solvent and recrystallization from 95 per cent ethanol there were obtained 4.02 grams
(74 per cent yield) of white crystalline 1-phenyl-1(2-thienyl)ethanol melting at 50 degrees Centigrade as reported by Thomas (45, pp.643-644).

4-Fluoro-3-methylacetophenone

4-Fluoro-3-methylacetophenone was prepared in the following manner: In a 250 milliliter three-neck flask fitted with a mechanical stirrer, separatory funnel and reflux condenser were placed ten grams o-fluorotoluene in 150 milliliters dry carbon disulfide. To this were added 26 grams anhydrous aluminum chloride and the mixture heated until gentle reflux began. 9.27 grams of acetic anhydride were then added slowly over 15 minutes' time. Gentle reflux was maintained for two hours longer. A condenser was then fixed to one of the side necks and the solvent removed. The contents of the flask were poured over 100 grams of ice to which 20 milliliters concentrated hydrochloric acid had been added. The mixture was extracted twice with 30 milliliter portions of ether and the ether washed twice with 30 milliliter portions of water, once with 35 milliliters 20 per cent sodium hydroxide and then twice more with 30 milliliter portions of water. The solution was dried one hour with five grams anhydrous calcium chloride and filtered. The solvent was then removed and the 4-fluoro-3-methylacetophenone distilled as a colorless liquid at 103 degrees.
Centigrade under a pressure of ten millimeters of mercury. 9.8 grams were obtained (71.0 per cent yield). Percentages of carbon and hydrogen were calculated to be 71.04 and 5.96; found 71.39 and 5.85. The 2,4-dinitrophenylhydrazone melted at 232.5-233.5 degrees Centigrade. Calculated percentages for carbon and hydrogen were 54.21 and 3.94; found 54.02 and 3.86.

It was deemed necessary to show that the structure of the 4-fluoro-3-methylacetophenone is as given. Accordingly, 200 milligrams of the compound were dissolved in five milliliters dioxane plus one milliliter of ten per cent sodium hydroxide. Iodine potassium-iodide solution made up as advised by Shriner and Fuson (42, pp.138-139) was added with shaking until a slight excess yielded a dark color of iodine at 60 degrees Centigrade. After crystallization of the iodoform was complete the mixture was filtered, decolorized with ten per cent sodium thiosulfate, acidified with dilute hydrochloric acid, and extracted with 30 milliliters ether. The ether layer was extracted with 30 milliliters ten per cent sodium hydroxide which was acidified with dilute hydrochloric acid to give .09 grams of white crystals which, when filtered and dried, melted at 164-165 degrees Centigrade given by Schiemann, et al. (39, p.745) as the melting point of 4-fluoro-3-methylbenzoic acid, showing that
the acylating agent attacks the position para to the fluorine atom in o-fluoro-toluene under the above conditions.

As further proof of the structure of 4-fluoro-3-methylacetophenone, a one gram portion of 4-fluoro-3-methylacetophenone was placed in a 500 milliliter three-neck flask fitted with stirrer, condenser, dropping funnel and heating unit. A saturated solution of potassium permanganate in water was added over a period of two hours until no more reduction of the oxidizing agent took place while under reflux. The mixture was filtered and the filtrate acidified with dilute hydrochloric acid causing white crystals to separate. These were filtered and redissolved in 20 milliliters of ten per cent sodium hydroxide and reprecipitated with dilute hydrochloric acid to give 0.7 grams of white crystals melting at 282-286 degrees Centigrade, as reported by Fosdick, et al. for 4-fluoroisophthalic acid (14, p.2309).

1-(4-Fluoro-3-methylphenyl)1-phenylethanol

1-(4-Fluoro-3-methylphenyl)1-phenylethanol was prepared as follows: In a 200 milliliter three-neck flask equipped with stirrer, reflux condenser and dropping funnel were placed .330 grams magnesium in 50 milliliters dry ether. To this were added slowly 2.17 grams bromobenzene
in 25 milliliters dry ether. After the magnesium had disappeared the flask was cooled and two grams of 4-fluoro-3-methylacetophenone in 25 milliliters dry ether were added slowly. On refluxing for one hour, the contents were poured on 50 grams of ice to which 20 milliliters water and five grams ammonium chloride had been added. The ether layer was washed twice with 20 milliliter portions of water, once with ten milliliters of ten per cent sodium hydroxide and twice more with 20 milliliter portions of water. On evaporation of the solvent the crystals were taken up in Skelly-solve F and recrystallized to give 2.6 grams of white 1-(4-fluoro-3-methylphenyl)1-phenylethanol (86 per cent yield) melting at 63 degrees Centigrade. Calculated percentages for carbon and hydrogen are 78.23 and 6.57; found 78.01 and 6.49.

1-(4-Fluoro-3-methylphenyl)1-phenylethylene

1-(4-Fluoro-3-methylphenyl)1-phenylethylene was prepared as follows: In a 250 milliliter three-neck flask fitted with stirrer, dropping funnel and reflux condenser were placed .640 grams of magnesium in 50 milliliters dry ether. To this were added slowly with stirring 4.24 grams bromobenzene in 50 milliliters dry ether. After the magnesium had disappeared, the flask was cooled and four grams
4-fluoro-3-methylacetophenone were slowly added. After refluxing 1.5 hours the contents were poured on 50 grams ice to which 20 milliliters of water and five grams ammonium chloride had been added. The ether layer was separated and washed twice with 20 milliliters water, once with ten milliliters ten per cent sodium hydroxide and again with the same amount of water. The solvent was removed, two milliliters 20 per cent sulfuric acid were added and the mixture refluxed one hour. The organic layer was separated and distilled at 172 degrees Centigrade under 20 millimeters pressure of mercury to give 3.34 grams of colorless liquid 1-(4-fluoro-3-methylphenyl)1-phenylethylene (60.2 per cent yield). Calculated percentages for carbon and hydrogen are 84.88 and 6.17; found 84.99 and 6.16.

4-Fluoro-3-methylbenzophenone

4-Fluoro-3-methylbenzophenone was prepared as follows: In a 25 milliliter flask was placed one gram of 1-(4-fluoro-3-methylphenyl)1-phenylethylene in ten milliliters glacial acetic acid. Two grams chromium trioxide were added and the solution refluxed for one hour. The contents were then cooled and poured into 100 milliliters of water. The crystals separating were filtered, washed well with water and taken up in ether. The ether layer was
washed with 20 milliliters of ten per cent sodium hydroxide and twice with 30 milliliters water. The solvent was removed and the solid recrystallized in Skelly-solve F to give 750 milligrams of white crystalline 4-fluoro-3-methylbenzophenone melting at 54 degrees Centigrade. The calculated percentages for carbon and hydrogen are 78.49 and 5.19; found 78.60 and 5.25.

Compound screening on mosquito larvae

The insect representative chosen for this study was the southern house mosquito larvae, *Culex quinquefasciatus* in the fourth instar. It is hardy and adaptable to laboratory conditions but perhaps less sensitive to toxic substances than other varieties.

The compounds were assayed in the following manner: Twenty milligrams of the compound were taken up in two milliliters of acetone and 198 milliliters water. By taking 0.15 and 1.5 milliliter aliquots and finally diluting to 15 milliliters with water the compounds were assayed at levels of both one and ten parts per million.

Round two-ounce bottles served as containers. Appropriate aliquots of sample were measured into the bottle and ten larvae were added in five milliliters of water. The volume was then diluted to 15 milliliters and
the bottles allowed to stand. The larvae were examined at 24 and 48 hour intervals, the criterion of death being lack of response to probing.

Results of this screening are listed in Table II, block (g) as per cent of colony dead after 24 hours' application of ten parts of compound per million of water.

Compound screening of microorganisms

The compounds under investigation in this study are quite water insoluble, and it was found to be very difficult to obtain reproducible results in experiments utilizing the photometer to assay bacteriostatic effects. The conventional method of screening compounds for bacteriostatic properties using the optical density of an inoculated culture media containing a known amount of compound for estimating cell growth has other undesirable features. It is laborious, time consuming and utilizes large amounts of chemicals when testing is attempted on a large scale.

For these reasons a gradient plate technique patterned after one reported by Bryson and Szybalski (5, pp.45-51) was investigated and developed as a tool for quick, approximate screening for large numbers of potential chemotherapeutics at a minimum of time and cost.

The method is described as follows: aseptic technique is used throughout, all glassware being sterilized
for 20 minutes at 15 pounds and the nutrient agar for ten minutes at 15 pounds pressure. To a plate nine centimeters in diameter placed at a slant as shown in Figure 1 are added 20 milliliters sterile nutrient agar so that the bottom is just covered.

Figure 1

After the agar has cooled and solidified, the plate is placed in the normal horizontal position and another 20 milliliter portion of sterile agar is added containing 200 micrograms penicillin G, potassium salt. See Figure 2.

Figure 2
The thickness of agar is found to be about 0.63 centimeters. After cooling two hours the agar plate is inoculated by making a streak approximately 0.5 centimeters wide with a sterile brush dipped in an inoculum of *Streptococcus fecalis* grown 48 hours in nutrient broth at 32 degrees Centigrade. The inoculum streak follows a line bisecting the upper wedge of agar. Finally the culture is incubated 18 hours at 32 degrees. It is then noted that the colonies have grown along the inoculum line beginning from the thin end of the top wedge of agar as shown in Figure 3.

Figure 3

Consider a section of the agar plate along the line inoculated. It is nine centimeters long, 0.63 centimeters high and about 0.5 centimeters wide as shown in Figure 4.
The section may be divided into 18 smaller sections each 0.5 centimeters in length with a volume of 0.1575 cubic centimeters. Section number one must contain approximately 1.575 micrograms of penicillin or 10 micrograms per milliliter, as this concentration was added in the top wedge initially. Due to downward diffusion, each successive section will be deprived of penicillin that will be estimated at one-eighteenth less than the preceding section.

By measurement it is determined that colonies of *S. faecalis* have stopped growing at a position designated by section number 16. Hence it is calculated that the colonies are inhibited by concentrations of penicillin of about 1.7 micrograms per milliliter, a figure within the range found by other methods (2, p.72). Any compound may be assayed in a similar manner.

In evaluating the accuracy one might expect from this assay it is to be remembered that no integration of concentration along the gradient is attempted. It is
assumed that diffusion downward reaches equilibrium. Outward diffusion is neglected. The merit of this method, however, is based on the excellent reproducibility of results and of the very rapid approximate evaluation of large numbers of potentially valuable compounds.

The values obtained by this method were compared with those found by standard dilution technique. Inoculated tubes containing ten milliliters of nutrient broth and varying levels of penicillin G, potassium salt were incubated at 32 degrees Centigrade for 18 to 24 hours. The growth was then estimated by means of a photometer with reference to uninoculated tubes. The results are tabulated in Table I.

**TABLE I**

**Smallest weight of penicillin G, potassium salt Required to completely inhibit growth**

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A total of 32 compounds were then screened by the gradient plate method and the results tabulated in Table II. Six microorganisms were chosen for screening with a view to obtaining as varied a representation as feasible. All organisms were obtained from stock cultures at Oregon State College and are as follows:

(a) *Escherichia coli* (sucrose negative)
(b) *Staphylococcus aureus* HSR9674; *Micrococcus pyogenes*
(c) *Streptococcus faecalis* ATCC1170
(d) *Saccharomyces ellipsoideus* (Burgundy wine)
(e) *Acetobacter suboxydans* ATCC621
(f) *Candida albicans*.

The values given in Table II are in micrograms required to completely inhibit growth. The highest level taken for assay was 250 micrograms per milliliter. The data is arranged in the following manner:

**SAMPLE TABLE II**

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>Structure</th>
<th>(a) E. coli</th>
<th>(b) M. pyogenes</th>
<th>(c) S. faecalis</th>
<th>(d) S. ellipsoideus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) A. suboxydans</td>
<td>(f) C. albicans</td>
<td>(g) <em>Culex quinquefasciatus</em></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Structure</th>
<th>&lt;14</th>
<th>112</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzophenone</td>
<td><img src="image1" alt="Structure Image" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzhydrol</td>
<td><img src="image2" alt="Structure Image" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-methylbenzhydrol</td>
<td><img src="image3" alt="Structure Image" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichlorobenzophenone</td>
<td><img src="image4" alt="Structure Image" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichlorobenzhydrol</td>
<td><img src="image5" alt="Structure Image" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>-</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>----------------------------------</td>
<td>---</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>1,1-bis-(p-chlorophenyl)ethanol</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td>35%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dimethoxybenzophenone</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dimethoxybenzohydrol</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,1-bis-(p-methoxyphenyl)ethanol</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichloro-3,3'-dinitrobenzophenone</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>% Conversion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>----------------------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichloro-3,3'-dinitrobenzhydrone</td>
<td><img src="image" alt="Structure" /></td>
<td>&lt;14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichloro-3,3'-diaminobenzophenone</td>
<td><img src="image" alt="Structure" /></td>
<td>not run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,4'-dichloro-3,3'-diaminobenzhydrol</td>
<td><img src="image" alt="Structure" /></td>
<td>not run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-chlorophenyl 2-thienyl ketone</td>
<td><img src="image" alt="Structure" /></td>
<td>not run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-chlorophenyl 2-thienylmethanol</td>
<td><img src="image" alt="Structure" /></td>
<td>70 112 70 42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE II (Continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>R</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenyl 2-thienyl ketone</td>
<td><img src="image1" alt="Structure" /></td>
<td>-</td>
<td>-</td>
<td>54</td>
</tr>
<tr>
<td>phenyl-2-thienyl-methanol</td>
<td><img src="image2" alt="Structure" /></td>
<td>-</td>
<td>192</td>
<td>-</td>
</tr>
<tr>
<td>1-phenyl-1-(2-thienyl)ethanol</td>
<td><img src="image3" alt="Structure" /></td>
<td>-</td>
<td>224</td>
<td>70</td>
</tr>
<tr>
<td>4,4'-bis-(dimethylamino)benzophenone</td>
<td><img src="image4" alt="Structure" /></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4,4'-bis-(dimethylamino)benzydrol</td>
<td><img src="image5" alt="Structure" /></td>
<td>-</td>
<td>-</td>
<td>42</td>
</tr>
</tbody>
</table>

Note: R, I, and R represent reaction conditions, with R indicating not run.
<table>
<thead>
<tr>
<th><strong>TABLE II (Continued)</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4-fluoro-3-methylbenzophenone</strong></td>
<td><img src="image1" alt="Chemical Structure" /></td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td><strong>4-fluoro-3-methylbenzhydrol</strong></td>
<td><img src="image2" alt="Chemical Structure" /></td>
</tr>
<tr>
<td></td>
<td>&lt;14</td>
</tr>
<tr>
<td><strong>1-(4-fluoro-3-methylphenyl)1-phenylethanol</strong></td>
<td><img src="image3" alt="Chemical Structure" /></td>
</tr>
<tr>
<td></td>
<td>42</td>
</tr>
<tr>
<td><strong>penicillin G, potassium salt</strong></td>
<td>13</td>
</tr>
<tr>
<td><strong>phenol</strong></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
DISCUSSION

In reviewing the data given in Table II regarding screening of the series of compounds on microorganisms, one fact is immediately apparent. Of the four types of bacteria studied not one was inhibited by a ketone. Phenyl 2-thienyl ketone in concentrations of 54 micrograms per milliliter inhibited the growth of the yeast \textit{S. ellipsoideus}, however. Apparently ketones of this type are relatively innocuous to the organisms studied.

In contrast to the ketones, reduction to the alcohol derivatives in general yielded highly toxic molecules. Of the nine ketones assayed, seven when reduced gave alcohols proving toxic in concentrations varying from less than 14 to about 225 micrograms per milliliter. Only the methoxy- and amino-chloro substituted derivatives failed to show toxic properties.

The unsubstituted benzhydrol inhibited the growth of both \textit{E. coli} and \textit{M. pyrogenese}, but those alcohols with additional substitution tended to prove toxic to a wider spectrum of organisms.

The \textit{\alpha}-methyl substituted benzhydrols showed varied effects. For benzhydrol itself toxicity was eliminated. For 4,4'-dichlorobenzhydrol, although toxicity was removed for the pathogenic yeast, two microorganisms were inhibited.
to a greater extent. The 4-fluoro-3-methylbenzhydrol was made more acceptable by the addition of an α-methyl group, but in contrast, the toxicity of phenyl-2-thienyl methanol was increased.

The fluoro-methyl substituted alcohols showed high toxicity exceeding that exhibited by the chloro derivatives. Addition of nitro groups to aid chlorine illustrated by 4,4'-dichloro-3,3'-dinitrobenzhydrol gave a molecule quite effective in hindering growth.

The thiophene ring substituted for phenyl gave a somewhat wider spectrum of activity, although in several cases the compound was somewhat less toxic.

Although an amino group in the 3 position as illustrated by 4,4'-dichloro-3,3'-diaminobenzhydrol was innocuous, the tertiary amino group in 4,4'-bis-(dimethylamino)benzhydrol yielded a compound quite effective for S. ellipsoideus and A. Suboxydans.

M. pyrogenes and S. ellipsoideus proved most susceptible, both being inhibited by eight compounds. C. albicans was most resistant, being affected by only 4,4'-dichlorobenzhydrol. This is to be noted, however, for this pathogenic yeast is extremely resistant to most chemotherapeutic agents.
Of the compounds listed in the addendum, 1,1-bis(4-chloro-3,5-dinitrophenyl)ethane proved to be quite toxic, as did the bis-(4-chlorophenyl)acetic acid.

Only three compounds proved to inhibit insects to any extent, the 4-chlorophenyl 2-thienyl ketone alone being investigated in this capacity for the first time.

Future investigation in this direction might be well spent determining the toxicity of these compounds on higher animals. If favorable results are obtained, further research pertaining to feasible alcohol derivatives of this general type might furnish valuable specific toxic agents of practical importance.

The gradient plate method for rapid screening of prospective chemotherapeutic agents shows great promise. Further development of this method would certainly be profitable.
SUMMARY

Following is a brief summary of this work:

1. A rapid gradient plate method of screening potential chemotherapeutic agents is reported.

2. Thirty-two compounds have been screened on four bacteria, two yeasts and one insect.

3. There is a definite contrast in the biological activity of the ketones and alcohols prepared, the former being nontoxic in the concentrations used.

4. Six new compounds plus derivatives are among those reported.
BIBLIOGRAPHY


ADDENDUM
ADDENDUM

**Bis-(p-chlorophenyl)methane**

*Bis-(p-chlorophenyl)methane* was prepared according to directions given by Smith, et al. (5, p.364). A theoretical yield melting at 55-56 degrees Centigrade was reported. A near theoretical yield melting at 55-56 degrees Centigrade was obtained.

**1,1-Bis-(p-chlorophenyl)ethylene**

*1,1-Bis-(p-chlorophenyl)ethylene* was prepared by the method given by Grummitt, et al. (2, pp.2265-2266). A yield of 88 per cent melting at 84-86 degrees Centigrade was reported. A yield of 86 per cent melting at 84-86 degrees Centigrade was found.

**1,1-Bis-(p-methoxyphenyl)ethylene**

*1,1-Bis-(p-methoxyphenyl)ethylene* was prepared according to the method given by Pfeiffer and Wizinger (3, p.144). A yield of 74 per cent was reported melting at 142-143 degrees Centigrade. A yield of 79.5 per cent melting at 142-143 degrees Centigrade was obtained.
**1,1-Bis-(p-dimethylaminophenyl)ethylene**

1,1-Bis-(p-dimethylaminophenyl)ethylene was prepared according to directions given by Pfeiffer and Wizinger (3, p.152). A 56 per cent yield was reported melting at 121-122 degrees Centigrade. A 59 per cent yield was found melting at 121-122 degrees Centigrade.

**1,1-Bis-(p-chlorophenyl)ethane**

1,1-Bis-(p-chlorophenyl)ethane was prepared according to the method of Grummitt, et al. (2, p.2266). A yield of 62 per cent melting at 54-55 degrees Centigrade was reported. A yield of 63 per cent melting at 54-55 degrees Centigrade was found.

**1,1-Bis-(p-methoxyphenyl)ethane**

1,1-Bis-(p-methoxyphenyl)ethane was prepared as follows: a mixture of 2.0 grams 1,1-bis-(p-methoxyphenyl)ethylene, 200 milliliters absolute alcohol and 200 milligrams copper-chromite catalyst was allowed to react with hydrogen in a bomb at 175 degrees Centigrade and 2000 pounds pressure. After two hours the bomb was removed. The solution was filtered hot, the solvent removed. The product was crystallized from 95 per cent alcohol to give 1.3 grams
(65 per cent yield) of 1,1-bis-(p-methoxyphenyl)ethane as reported by Price and Mueller for this compound (4, p.636).

1,1-Bis-(4-chloro-3,5-dinitrophenyl)ethane

1,1-Bis-(4-chloro-3,5-dinitrophenyl)ethane was prepared according to the method given by Forrest, et al. (1, p.338). A product melting at 261 degrees Centigrade was reported. A yield of 52 per cent melting at 261 degrees Centigrade was found.

Bis-(p-chlorophenyl)acetic acid

Bis-(p-chlorophenyl)acetic acid was prepared according to directions given by Smith, et al. (5, pp.364-365). There was reported a 35.3 per cent yield which melted at 167.5-168 degrees Centigrade. There was found a 39.6 per cent yield which after crystallization from 36 per cent acetic acid melted at 164 degrees Centigrade.

Compound screening

The above compounds were treated for screening exactly as before, and the results are listed in Table III precisely as in Table II.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Retention Time</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1-bis-(p-chlorophenyl)ethane</td>
<td><img src="image1" alt="Structure" /></td>
<td>-</td>
<td>not run 57%</td>
</tr>
<tr>
<td>1,1-bis-(p-methoxyphenyl)ethane</td>
<td><img src="image2" alt="Structure" /></td>
<td>-</td>
<td>not run -</td>
</tr>
<tr>
<td>1,1-bis-(4-chloro-3,5-dinitrophenyl)ethane</td>
<td><img src="image3" alt="Structure" /></td>
<td>112 &lt;14 &lt;14</td>
<td>112 - -</td>
</tr>
<tr>
<td>bis-(p-chlorophenyl)acetic acid</td>
<td><img src="image4" alt="Structure" /></td>
<td>-</td>
<td>&lt;14 &lt;14 -</td>
</tr>
<tr>
<td>1-(4-fluoro-3-methylphenyl) 1-phenylethylene</td>
<td><img src="image5" alt="Structure" /></td>
<td>-</td>
<td>not run -</td>
</tr>
</tbody>
</table>

TABLE III
<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis-(p-chlorophenyl)methane</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
</tr>
<tr>
<td>1,1-bis-(p-chlorophenyl)ethylene</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
</tr>
<tr>
<td>1,1-bis-(p-methoxyphenyl)ethylene</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
</tr>
<tr>
<td>1,1-bis-(p-dimethylaminophenyl)ethylene</td>
<td><img src="image" alt="Structure" /></td>
<td>-</td>
</tr>
</tbody>
</table>
ADDENDUM

BIBLIOGRAPHY


