more effective than commercial penicillin against a strain of *Klebsiella pneumoniae* type A and a strain of *Bacillus cereus*. No difference in effect could be shown, however, on four strains of *Staphylococcus aureus*. The same authors[1] have shown penicillin X to be more effective in protecting mice against type I pneumococci and in the treatment of gonorrhea in humans.

This report shows the concentration of penicillin G and penicillin X in units and micrograms per ml necessary to inhibit the growth of strains of the following organisms: *Staphylococcus aureus, Bacillus subtilis,* types I, II and III pneumococci, groups A, B and D streptococci, *Erysiphelothrix rhusipathiae* and *Escherichia coli.*

Experimental: A chloroform-extracted calcium salt of penicillin, 1,050 units per mgm, was used throughout for determining the activity of penicillin G. Two crystalline sodium salts of penicillin X,[2] 846 units and 990 units per mgm, respectively, were used for comparative purposes. Sterile penicillin solutions were obtained by filtration through Seitz filters. Serial dilutions of penicillin were made with sterile A.C. or brain-heart-infusion broth (Difco). The cultures to be tested for the activity of penicillins G and X were grown in A.C. or brain-heart-infusion broth in a 37° C water bath for about 3 hours, or until they had reached the logarithmic growth phase. One milliliter of a one to ten dilution of the culture was then inoculated into 1-ml volumes of the serial dilutions of penicillin. These experimental cultures were incubated at 30° C for 18 hours. The results are recorded as the lowest concentration of penicillin G or X that prevented the visual appearance of growth at the end of 18 hours' incubation. *Results:* The first column of Table 1 shows the number of organisms in millions per ml, as indicated by plate counts, at zero time. Columns 2 and 3 show the concentration of penicillin G or X in units per ml of medium required to inhibit visible growth for a period of 18 hours' incubation at 30° C. Table 2 shows the concentration of penicillin G or X in micrograms of penicillin per ml required to inhibit the appearance of visible growth. The final columns in Tables 1 and 2 show the comparative activity of penicillins G and X, as expressed by the ratio of the units or micrograms of penicillin G to penicillin X to inhibit growth.

TABLE 1

UNITS OF PENICILLIN PER ML TO INHIBIT GROWTH

Organism	Inoculum millions/ml	Units* penicillin G	Units* penicillin X	Ratio $\frac{G}{X}$
Staphylococcus aureus— NRRL–313	1.4	.0625	.0625	1.0
Bacillus subtilis—3R9675	1.3	.0982	.0982	1.0
Pneumococcus Type I (SVI)	1.7	.03125	.0156	2.0
Pneumococcus Type II	0.7	.01225	.0050	2.4
" " III	0.5	.0121	.0050	2.4
Streptococcus Group D	0.4	4.0000	1.7000	2.4
" " B	1.0	.2030	.0664	3.0
" " A	0.6	.0176	.0063	2.8
Erysiphelothrix rhusipathiae	1.7	.1607	.0491	3.3
Escherichia coli	1.7	133.9000	46.8750	2.9

*Mean values for 7 trials carried out on different days.

TABLE 2

MICROGRAMS* OF PENICILLIN PER ML TO INHIBIT GROWTH

Organism	Penicillin G	Penicillin X	Ratio $\frac{G}{X}$
Staphylococcus aureus— NRRL–313	.040	.060	0.7
Bacillus subtilis—3R9675	.059	.098	0.6
Pneumococcus Type I (SVI)	.019	.016	1.2
" " II	.007	.005	1.4
" " III	.007	.005	1.4
Streptococcus Group D	2.400	1.700	1.4
" " B	.120	.066	1.8
" " A	.010	.006	1.7
Erysiphelothrix rhusipathiae	.097	.049	2.0
Escherichia coli	81.000	46.900	1.7

* Calculated on the basis of 1,650 units/mgm for pure penicillin G and 1,000 units/mgm for pure penicillin X.

Discussion: In agreement with Welch *et al.*[1] penicillin G and X appear equally effective on a unit basis in inhibiting the growth of *Staphylococcus aureus;* likewise, both penicillins are equally effective against the strain of *Bacillus subtilis* used. On a weight basis, however, penicillin G is more effective than penicillin X on the strains of these two organisms used in this study.

For the remaining organisms tested, penicillin X is more effective both on a unit and on a weight basis than is penicillin G in inhibiting growth *in vitro.* Depending on the organisms tested, 2 to 3 times more penicillin G than penicillin X is required on a unit basis to inhibit visible growth, while on a weight basis 1.2 to 2 times more G than X is necessary.

Summary: The data presented indicate that penicillin X is more effective than penicillin G against a number of different organisms.

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[2] The sodium salt of penicillin X used in these experiments was prepared by E. F. Williams, of the Physics Research and Testing Division of these laboratories.

THE SPECIFICITY OF THE XANTHYDROL-PYRIDINE REACTION FOR 2, 2 BIS (p-CHLOROPHENYL) 1,1,1 TRICHLOROETHANE (DDT)

STIFF and Castillo[1] reported a new sensitive colorimetric test for 2,2 bis (p-chlorophenyl) 1,1,1 trichloro-

ethane (DDT) based upon the formation of a red color by heating DDT with anhydrous pyridine solution containing xanthydrol and solid KOH. The reaction is sensitive to as little as 10 micrograms and is quantitative within the range of 10–240 micrograms of DDT. No mention was made of the specificity of this reaction.

In view of the studies on the metabolism of DDT and its analogues which are being carried out in this laboratory it is of interest to examine the behavior of the various analogues and derivatives of DDT towards Stiff and Castillo's new reaction. The results of the test applied to eighteen compounds are shown in Table 1.

TABLE 1

THE REACTION OF DDT, ITS DERIVATIVES AND ANALOGUES WITH XANTHYDROL-PYRIDINE; THE ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF THE POSITIVE REACTIONS

	Compound	Reaction	Absorption max. mμ	Extinction coefficient
(1)	$(p\text{-}ClC_6H_4)_2CHCCl_3$	+	500	10.4×10^3
(2)	$(p\text{-}ClC_6H_4)(C_6H_5)CHCCl_3$	+	500	9.2×10^3
(3)	$(p\text{-}ClC_6H_4)_2CHCBr_3$	+	506	2.9×10^3
(4)	$(p\text{-}BrC_6H_4)_2CHCCl_3$	+	520	10.8×10^3
(5)	$(C_6H_5)_2CHCCl_3$	+	495	3.1×10^3
(6)	$(p\text{-}CH_3C_6H_4)_2CHCCl_3$	+	488	2.5×10^3
(7)	$(p\text{-}CH_3OC_6H_4)_2CHCCl_3$	+	485	2.1×10^3
(8)	$(p\text{-}C_2H_5OC_6H_4)_2CHCCl_3$	+	485	1.4×10^3
(9)	$(p\text{-}C_3H_7OC_6H_4)_2CHCCl_3$	+	480	2.0×10^3
(10)	$(p\text{-}C_4H_9OC_6H_4)_2CHCCl_3$	+	482	2.0×10^3
(11)	$(p\text{-}ClC_6H_4)_2C=CCl_2$	+	500	6.6×10^3
(12)	$(C_6H_5)_2C=CCl_2$	+	490	1.9×10^3
(13)	$(p\text{-}BrC_6H_4)_2C=CCl_2$	+	482	1.5×10^3
*(14)	$(p\text{-}HOC_6H_4)_2CHCCl_3$	+	–	–
†(15)	$(p\text{-}CH_3COOC_6H_4)_2CHCCl_3$	+	–	–
(16)	$(p\text{-}ClC_6H_4)_2CHCOOH$	–	–	–
(17)	$(p\text{-}ClC_6H_4)_2CH_2$	–	–	–
(18)	$(p\text{-}ClC_6H_4)_2C=O$	–	–	–

* Compound No. 14 gave a dirty reddish violet color with bluish fluorescence.
† Compound No. 15 gave a reddish violet color and then a precipitate of brownish red.

EXPERIMENTAL

The compounds[2] used in Table 1 were weighed on the basis of 5.65×10^{-7} moles per 5 cc of anhydrous ether. Five cc of each solution were pipetted into individual test tubes 16 × 150 mm, and the colorimetric reaction as described by Stiff and Castillo was carried out on each tube in duplicate after evaporation of the ether. As pointed out by these authors, the presence of water and moisture has an inhibiting effect on color formation. We found this to be true, especially on hot humid days. Consistent results were obtained, however, by the introduction of a pellet of KOH to the tube containing 2 cc of the reagent and 4 cc of pyridine during the heating and development of color. The visible absorption curves of those compounds giving a positive reaction were obtained in a Coleman Spectrophotometer Model 11 with an effective width of 35 mμ. The extinction coefficient at 500 mμ of each of the various compounds were calculated from absorption measurements made in the same instrument in 1 cm thick absorption cells. The concentration of each compound in the reaction was such that light transmitted under this condition was approximately 50 per cent. of the incident light.

DISCUSSION

From the data in Table 1 it is seen that all compounds giving a positive reaction contain aliphatic halogen. Compounds having the structure $> CHCX_3$ or $> C=CX_2$ (X = Cl or Br) in all likelihood would give a positive reaction. On a molar concentration the strongest colors in descending order are Compounds (4), (1), (2) and (11). With the exception of compounds (4) substitution of the Cl in the para position in DDT by other groups decreases the color intensity. Likewise the substitution of Br in the side chain decreases the color intensity. The complete removal of the halogen from the aliphatic part of the molecule renders the compound negative in the reaction.

In view of these observations made on eighteen compounds the xanthydrol-pyridine reaction has no specificity for DDT.

SUMMARY

The Stiff and Castillo colorimetric test for DDT was extended to eighteen analogues and derivatives of DDT. The absorption maxima and extinction coefficients of the colored reactions were also obtained. The test is not specific for DDT. Of the compounds tested, the reaction was given by those having the structure $> CHCX_3$ or $> C=CX_2$.

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[1] H. A. Stiff and J. C. Castillo, SCIENCE, 101: 440, 1945.

[2] Compounds Nos. 1, 2, 3 and 13 were kindly furnished by Dr. H. L. J. Haller, of the Department of Agriculture. Compound No. 18 was obtained from Eastman Kodak Company. Compounds Nos. 16 and 17 were supplied by Dr. T. R. Sweeney of this laboratory. The rest of the compounds were synthesized by one of us (N.E.S.) following published material.

PHYSIOLOGICAL EVIDENCE OF A SITE OF ACTION OF DDT IN AN INSECT

WHEN DDT (1-trichloro-2,2-bis (*p*-chlorophenyl) ethane) solution[1] is injected into a roach, *Periplaneta*

[1] The following definitions apply to this paper: DDT solution, a high concentration (*ca.* 10 per cent.) of DDT in corn oil. Nicotine solution, a high concentration (*ca.*