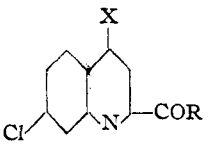
TABLE I

QUINALDIC ACID DERIVATIVES,



No.	X	R	M. p., °C.[a]	Formula	Analyses, % Calcd.	Found
I	Cl	OH	182–183	$C_{10}H_5Cl_2NO_2$	N, 5.79	5.76
II	Cl	OC_2H_5	77.5–78.5	$C_{12}H_9Cl_2NO_2$	N, 5.19	5.11
III	Cl	NH_2	288–290	$C_{10}H_6Cl_2N_2O$	N, 11.62	11.83
IV	OC_2H_5	NH_2	201–202	$C_{12}H_{11}ClN_2O_2$	C, 57.49	57.44
					H, 4.42	4.21
V	Cl	$N(C_2H_5)_2$	107–108	$C_{14}H_{14}Cl_2N_2O$	Cl, 23.86	23.80
VI	Cl	[b]	69–70[c]	$C_{16}H_{18}Cl_2N_2O_2$	Cl, 20.78	20.60
VII	Cl	[d]	82–83[e]	$C_{16}H_{18}Cl_2N_3O$	N, 12.35	12.52
VIII	OC_2H_5	[d]	65–66[f]	$C_{18}H_{24}ClN_3O_2$	N, 12.01	12.29
IX	OC_3H_7-*n*	[d]	66–67	$C_{19}H_{26}ClN_3O_2$	N, 11.55	11.31
X	OC_4H_9-*n*	[d]	68–69	$C_{20}H_{28}ClN_3O_2$	N, 11.12	10.80

[a] All melting points are uncorrected. [b] R = $OCH_2CH_2N(C_2H_5)_2$. [c] Hydrochloride from dilute hydrochloric acid, m. p. 233–234°; calcd. N, 7.42; found: N, 7.35. [d] R = $NHCH_2CH_2N(C_2H_5)_2$. [e] Hydrochloride from isopropyl alcohol, m. p. 230–231°; calcd. N, 11.16: Cl^-, 9.41; found: N, 11.20; Cl^-, 9.36. [f] Hydrochloride from alcohol–acetone–ether mixture, m. p. 178–179° d.; calcd. N, 10.88; Cl^-, 9.18; found: N, 11.30; Cl^-, 8.86.

Treatment of either 4,7-dichloroquinaldic acid (I) or its ethyl ester (II) with sodium ethylate failed to give the desired 4-ethoxy derivatives. Similar results were obtained starting with the basic ester, diethylaminoethyl 4,7-dichloroquinaldate (VI). The sodium salt of the free acid, which apparently formed in each instance, was too insoluble to react. However, the basic amide, N-diethylaminoethyl 4,7-dichloroquinaldamide (VII), prepared from the dichloro ester (II) by heating with N,N-diethylethylenediamine, did react with the appropriate sodium alkoxide to give the 4-alkoxyquinaldamides, VIII, IX and X.

Preliminary investigation indicated that N-diethylaminoethyl 7-chloro-4-ethoxyquinaldamide (VIII) had little if any local anesthetic activity at a concentration of 1% when tested on the external canthus of rabbits. Compounds IX and X, the 4-propoxy and 4-butoxyquinaldamides, are too insoluble in the desired *p*H range to be of any practical value as local anesthetics.

Experimental

Preparation of 4,7-Dichloroquinaldic Acid (I) and Ethyl Ester (II).—The appropriate 7-chloro-4-hydroxyquinoline derivative (5 g.) was refluxed for one hour with 30 ml. of phosphorous oxychloride. After cooling, the solution was poured into ice-water and the product was filtered off, washed with water, dried and recrystallized from toluene for I and ethanol for II. The yields were approximately 70%.

Preparation of 4,7-Dichloroquinaldamide (III).—Ammonia was bubbled into a stirred solution of 20 g. of ethyl 4,7-dichloroquinaldate in 200 ml. of absolute ethanol. The crude amide which separated in practically quantitative yield, was recrystallized from butanol.

Preparation of 7-Chloro-4-ethoxyquinaldamide (IV).—Twelve grams of the amide (III) was added to an excess of sodium ethylate in absolute ethanol and refluxed with stirring for two hours. The solid which remained undissolved was filtered off and recrystallized from acetic acid to give 5.5 g. of starting amide. Two grams of material was obtained from the filtrate melting at 190–192°. The product was recrystallized from acetic acid and then from ethanol.

Preparation of 4,7-Dichloro-N-diethylquinaldamide (V) and 4,7-Dichloro-N-(2-diethylaminoethyl)-quinaldate (VI).—The dichloro acid (I) in five volumes of xylene was refluxed with two volumes of thionyl chloride for two hours and the solvent removed *in vacuo*. The residue was suspended in dry benzene and refluxed for one hour with diethylamine (for V) or diethylaminoethanol (for VI) and poured into water. The solution was made alkaline with sodium carbonate solution and extracted with ether. Removal of the ether in the case of V gave the crude diethylamide. For the basic ester (VI) the ether was dried and the hydrochloride prepared. After purification it was converted to the base which was then recrystallized from Skellysolve A.

Preparation of 4,7-Dichloro-N-(2-diethylaminoethyl)-quinaldamide (VII).—A mixture of 56.8 g. of the dichloro ester (II), 35 g. of N,N-diethylethylenediamine and 350 ml. of ligroin (b.p. 100–140°) was refluxed for four hours and the solvent removed by distillation. The residue was dissolved in hot ligroin (b.p. 60–68°), filtered with charcoal, and the filtrate cooled to give 60 g. of crude amide (VII), m.p., 73–76°. The product was purified *via* its hydrochloride and then recrystallized from ligroin (b.p. 28–38°).

Preparation of Compounds VIII, IX and X.—The basic amide, VII (0.04 mole) was added to a solution of sodium (0.05 mole) in 100 ml. of the appropriate alcohol. After refluxing for one-half hour, the solvent was distilled and the residue taken up in water and ether extracted. The ether was dried and removed to yield the crude 4-alkoxyquinolines. The purified products from ligroin (b.p. 28–38°) were obtained in approximately 30% yields.

STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, NEW YORK RECEIVED MARCH 14, 1949

The Use of Counter-Current Distribution for the Characterization of Streptomyces Antibiotics[1]

BY E. AUGUSTUS SWART

The search for new antibiotics produced by various species and strains of actinomycetes has often led to the isolation of substances previously

(1) Supported by a grant from the Commonwealth Fund.

described in the literature. Occasionally the clear-cut identity of these new substances with known preparations is ambiguous. Recently, in this Laboratory, streptothricin VI, hereinafter referred to as S VI, was isolated from a culture filtrate of *Streptomyces lavendulae* 3516.[2] S VI gave the same qualitative bacterial spectrum as streptothricin but the quantitative effects on some of the microörganisms tested were different; S VI also appeared to show a lower toxicity than streptothricin. To characterize these two crude substances, recourse was had to the use of the 24-plate Craig Counter-Current Distribution Method,[3] whereby a distribution curve obtained for a given substance would show only one peak if there were but one antimicrobial substance present.

Plaut and McCormack[4] have distributed the streptomycin complex in the Craig machine between bicarbonate buffer and pentasol, using stearic acid as a carrier. O'Keeffe, Dolliver, and Stiller[5] have succeeded in extracting streptomycin into pentasol from a borate–phosphate buffer using lauric acid as a carrier.

In this Laboratory, streptothricin from several sources and S VI have been distributed in the Craig machine between borate buffer and pentasol, using stearic acid as the carrier.[6] To prepare the two liquid phases required for the distribution, 0.5 *M* boric acid in 0.5 *M* potassium chloride was adjusted, by addition of the required volumes of 0.5 *M* sodium hydroxide, to *p*H 7.35–7.50, 7.80–8.00 or 8.30–8.50, depending on the stearic acid used in the next step. One of these buffers was then shaken to equilibrium with an equal volume of 5% (w./v.) stearic acid in pentasol to yield, respectively, systems having a *p*H of 7.3, 7.6 or 7.8. The aqueous phase was used as the lower layer and the pentasol phase as the upper layer in the Craig machine. A sample of test material, equivalent to 3,000–5,000 units,[7] was dissolved in the aqueous phase of tube 0 in the Craig machine. Equilibration of the phases was effected by rotating the machine for two minutes at 27 revolutions per minute. After two minutes of standing, the upper half of the machine was moved to the next position. After a twenty-four plate distribution with 8-ml. layers, the upper and lower layers of a given tube were sucked into a 25-ml. separatory funnel, acidified to *p*H 1.5–2.0 by addition of 2 drops of concentrated hydrochloric acid, and shaken for five minutes with 5 ml. of benzene to displace the antibiotic into the lower layer. Five-ml. aliquots of the aqueous layers were concentrated *in vacuo* at 25–30° to remove organic solvents. The concentrates were diluted to 5 ml. with distilled water and bio-assayed. The concentrations in the lower layers were plotted as ordinates against the tube numbers to obtain the distribution curves shown in the figures.

Distribution curves, obtained by the above procedure, showed only one peak for streptothricin, S VI and streptomycin. Distribution coefficients for these substances were calculated by the second method of Williamson and Craig.[8] Table I summarizes the results obtained in these distributions; "tube" signifies that cell in the Craig machine where the maximum potency occurred, and *K* is the calculated distribution coefficient.

TABLE I

Distributed solid	Potency of distributed solid, u./mg.	*p*H 7.3 Tube	*p*H 7.3 *K*	*p*H 7.6 Tube	*p*H 7.6 *K*	*p*H 7.8 Tube	*p*H 7.8 *K*
Streptothricin	195					14	1.40
	250					14	1.36
	342	2	0.12	8	0.57	14	1.40
	800	2	.12			14	1.36
S VI	200	2	.12	8	.56	14	1.37
	491	2	.12	8	.57	14	1.38
Streptomycin	716	3	.16	9	.64	16	2.05

Streptothricin and S VI also showed the same maxima when they were distributed in the system of Plaut and McCormack; the calculated distribution coefficient for these substances was 0.54. The distribution curves for streptothricin, S VI and a mixture of the two are given in Fig. 1. Though the peaks are at different heights, due to different amounts of initial material used, they all occur at tube 8. Hence, streptothricin and S VI are apparently identical.

The data presented in Table I illustrate the difficulty of differentiating between streptothricin and streptomycin in the three borate buffer systems studied. Though the distribution coefficients of both substances vary with *p*H, in agreement with the results reported by Craig, *et al.*,[9] for bases of the plasmochin type, they are approximately the same at each *p*H used. On the other hand, the distribution coefficients of the two antibiotics are vastly different in the system of Plaut and McCormack. Streptothricin showed a peak in the distribution curve at tube 8, corresponding to a calculated distribution

(2) D. Hutchison, E. A. Swart and S. A. Waksman, *Arch. Biochem.*, in press.

(3) L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944).

(4) G. W. Plaut and D. R. McCormack, THIS JOURNAL, **71**, 2264 (1949).

(5) A. E. O'Keeffe, M. Dolliver and E. T. Stiller, *ibid.*, **71**, 2452 (1949).

(6) Baker and Adamson stearic acid, USP grade, code number 1172, supplied by the General Chemical Company, has been used in these experiments. When it was noted that different lots of this material gave different distribution coefficients for a given antibiotic, it was decided to use as a standard of reference the results obtained by Plaut and McCormack for streptomycin. Stearic acid that gave a peak at tube 21 in the distribution curve for streptomycin, in the system of Plaut and McCormack, was used in this investigation.

(7) S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exptl. Biol. Med.*, **49**, 207 (1942).

(8) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).

(9) L. C. Craig, C. Golumbic, H. Mighton and E. J. Titus, *J. Biol. Chem.*, **161**, 321 (1945).

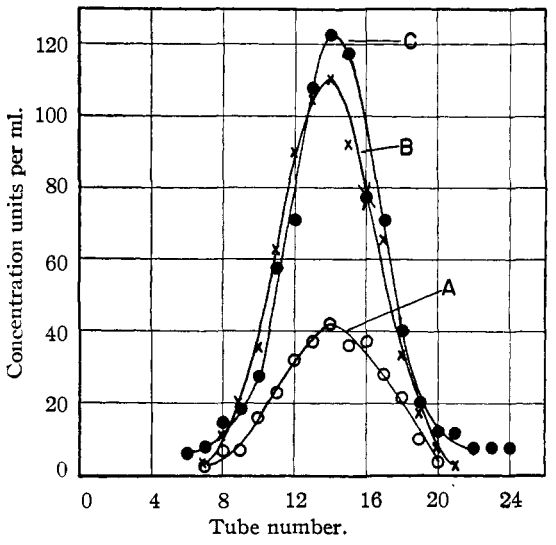


Fig. 1.—Distribution patterns between borate buffer at *p*H 7.8 and pentasol: A, 3450 units of streptothricin; B, 4095 units of S VI; C, a mixture of 1735 units of streptothricin and 1810 units of S VI.

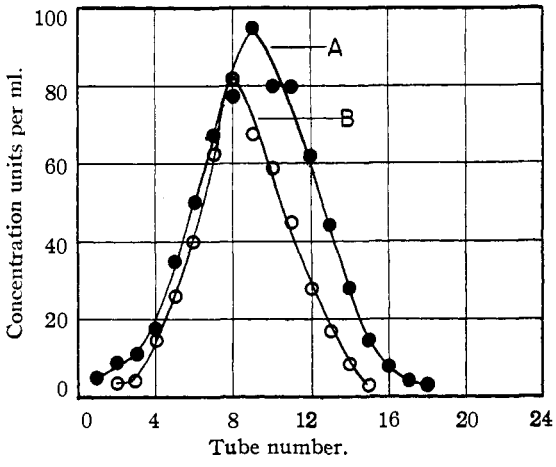


Fig. 2.—Distribution patterns between borate buffer at *p*H 7.6 and pentasol: A, 4650 units streptomycin; B, 3440 units streptothricin.

coefficient of 0.54. Streptomycin showed a peak at tube 21 with a corresponding distribution coefficient of 6.38. The distribution curves for streptothricin, streptomycin, and a mixture of these two antibiotics are shown in Fig. 3. It is interesting to note that the distribution of streptomycin in the bicarbonate buffer is considerably different from that in borate buffer at the same *p*H (7.6), while the distribution of streptothricin is the same in both systems. This might be explained on the basis of interaction of borate with the sugar moiety of streptomycin. The importance of using more than one system to characterize an antibiotic by the distribution analysis technique is clearly demonstrated by these results.

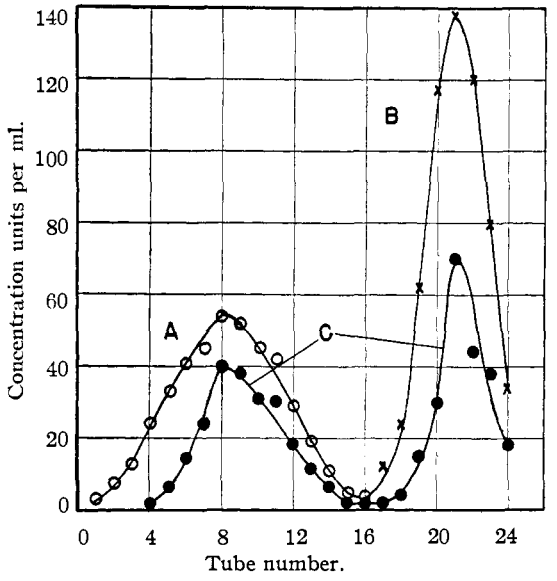


Fig. 3.—Distribution patterns between bicarbonate buffer and pentasol: A, 3630 units streptothricin; B, 4933 units streptomycin; C, a mixture of 2160 units of streptothricin and 2427 units streptomycin.

Thanks are due to Commercial Solvents Corporation, to Dr. O. Wintersteiner of the Squibb Institute for Medical Research, and to Dr. R. L. Peck of Merck and Company for supplying samples of streptothricin used in this work; to Dr. E. T. Stiller of the Squibb Institute for Medical Research for supplying the stearic acid used as a standard; and to Miss Dorris Hutchison for carrying out the bio-assays. The author also wishes to express his appreciation and thanks to Dr. Selman A. Waksman for helpful coöperation in carrying out this investigation.

This method is being investigated further as a possible means of characterizing other antibiotics produced by actinomycetes.

DEPARTMENT OF MICROBIOLOGY
NEW JERSEY AGRICULTURAL EXPERIMENT STATION
RUTGERS UNIVERSITY—THE STATE
UNIVERSITY OF NEW JERSEY
NEW BRUNSWICK, NEW JERSEY

RECEIVED FEBRUARY 12, 1949

The *trans* Isomer of 4-Amino-4′-hydroxy-α,α′-diethylstilbene

BY PHILIP WEISS

In a publication from this Laboratory,[1] the preparation of 4-amino-4′-hydroxy-α,α′-diethylstilbene was described and its melting point was given as 155–156°. From a crude sample which was prepared at that time I have now obtained by several recrystallizations from methanol a compound which melts at 180–183°.[2]

(1) M. Rubin and H. Wishinsky, THIS JOURNAL, 66, 1948 (1944).
(2) All melting points are uncorrected.