could be observed in the region of the solvent front. This paper gave erratic results.[12]

To determine the extent of impregnation of the paper by these methods, various samples of the treated paper were ashed and the residues assayed for chromium with the following results[13]:

Sample	Cr(mg./25 cm.²)
Dipped, soln. A	0.26; 0.23
Dipped, soln. B	.24; .24
Sprayed, soln. B	.24; .22

Solutions A and B refer to solutions 2% in both "Quilon" and neutralizer which were prepared at different times.

Chromatography.—Reagent grade solvents were used throughout. All ratios of solvents represent percentages by volume. In all cases 10 γ of material was applied to a spot 1.5 cm. in diameter on a strip of paper about 4 cm. wide. The papers were suspended from metal troughs in large test-tubes (7 × 50 cm.) which contained a few cc. of the solvent mixture and which were sealed with rubber stoppers during the course of chromatography. Generally two papers were suspended from each trough. Descending chromatography was used throughout, the solvent being allowed to run 25–35 cm. from the origin. The papers were allowed to dry thoroughly before testing for the presence of the material being chromatographed. R_f values were measured from the foremost point of the origin and the leading portion of the spot.

As the percentage of water in the solvent was increased the time required for the solvent to run the specified distance was also greatly increased. The upper limit of dilution for methanol is about 20%. Methanol–water 75:25 takes very long to run and at lower temperatures does not wet the paper. Methanol–water 7:3 will not wet the paper. The dilution limit is somewhat higher with the higher alcohols.

Detection of Steroids.—The methods available for the detection of steroids on "Quilon" treated paper have already been reported.[14] In general, iodine vapor was used for detection of the androgens and progesterone; silicotungstic acid for cholesterol, epicholesterol, cholestanol, stigmasterol and cholestenone; antimony pentachloride for sitosterol, ergosterol and 7-dehydrocholesterol; and triphenyl tetrazolium chloride[3] for the corticosteroids. In some cases, several methods were used for detection of the same steroids and the same R_f values were obtained. With cholesterol-4-C^{14} and epicholesterol-4-C^{14} the color tests were further confirmed by radioautography.[15]

Acknowledgment.—The authors are deeply grateful to Prof. Melvin Calvin for many helpful discussions during the course of this work. Thanks are also due to E. I. du Pont de Nemours and Company, Inc., for their generous gifts of the various impregnating agents; to the Ciba Pharmaceutical Products, Inc., for the androgens; to Dr. E. C. Kendall for cortisone; to the Upjohn Company for the other corticosteroids; to Prof. W. G. Dauben for cholestanol and cholestenone; to Dr. J. F. Eastham for cholesterol-4-C^{14} and epicholesterol-4-C^{14}; and to the Chemical Specialties Company, Inc., for the progesterone and pregnenolone used in these experiments.

(12) The other chromic chloride complexes were all mixed with "neutralizer" prior to treatment of the paper.

(13) Analyses by Mr. V. Tashinian of the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

(14) D. Kritchevsky and M. R. Kirk, *Arch. Biochem. Biophys.*, **35**, 346 (1952).

(15) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY OF THE ALTON OCHSNER MEDICAL FOUNDATION AND THE DEPARTMENT OF BIOCHEMISTRY, TULANE UNIVERSITY SCHOOL OF MEDICINE]

Arylnitroalkenes: A New Group of Antibacterial Agents[1]

BY OTTO SCHALES AND HEINZ A. GRAEFE

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It was found that β-nitrostyrene inhibits bacterial growth and that its effectiveness was only slightly reduced when the culture medium contained protein. The influence of structural variations on antibacterial activity was studied by preparing and testing a total of 55 compounds, including 20 arylnitroalkenes which had not been described before. 4-Hydroxy-β-nitrostyrene, previously prepared by a cumbersome route in poor yield, was obtained in 79% yield when a mixture of equimolecular amounts of aldehyde and nitromethane was heated in presence of aniline as catalyst. Most effective amongst the substances tested were 1-(3,4-dichlorophenyl)-2-nitropropene against *Micrococcus pyogenes* var. *aureus* in protein-free medium, 2,6-dichloro-β-nitrostyrene against the same organism in presence of albumin, and 1,4-bis-(β-nitrovinyl)-benzene against *Escherichia coli*. Intravenous administration to mice of a few selected compounds showed that these substances were not very toxic.

It was found that β-nitrostyrene in concentrations of 1 mg. or less per 100 ml. of culture medium inhibited the growth of *Micrococcus pyogenes* and of *Escherichia coli* and that its effectiveness was only slightly reduced when albumin was added to the culture medium. Earlier reports dealing with the biological activity of β-nitrostyrene stated that it had a detrimental effect on insects[2,3] and on the growth of fungi[2,4,5] and that it could be used for the protective treatment of textiles, leather and other organic materials.[2] A comparison of a few nitrostyrenes showed that there was no correlation between the physiological effect on man and fungistatic activity.[4] There was little difference, for example, in the fungistatic effectiveness of β-nitrostyrene and of 4-methoxy-β-nitrostyrene, but in man the first compound acted as a powerful sternutator (an irritant which provokes

(1) Presented before the Division of Biological Chemistry at the 121st Meeting of the American Chemical Society in Milwaukee, Wisconsin, March 31, 1952.

(2) E. W. Bousquet, J. F. Kirby and N. E. Searle, U. S. Patent 2,335,384 (Nov. 30, 1943).

(3) A. W. A. Brown, D. B. W. Robinson, H. Hurtig and B. J. Wenner, *Can. J. Research*, **26D**, 177 (1948).

(4) P. W. Brian, J. F. Grove and J. C. McGowan, *Nature*, **158**, 876 (1946).

(5) J. C. McGowan, P. W. Brian and H. G. Hemming, *Ann. Applied Biol.*, **35**, 25 (1948).

sneezing) whereas the methoxy derivative was non-irritant.[4]

Since the antibacterial activity of β-nitrostyrene had not been noted before,[5a] it seemed of interest to prepare a variety of related compounds and to study the influence of structural variations on antibacterial effectiveness. A total of 55 substances was synthesized, including 20 arylnitroalkenes which had not been described before. Of the substances tested, 32 were obtained by condensing nitromethane and 23 by condensing nitroethane with various aldehydes.

Synthetic Methods.—The condensations of nitromethane and nitroethane with aromatic and heterocyclic aldehydes were carried out with one or the other of two general methods and their modifications.

Method A.—In this procedure, first described by Thiele[6] and later modified by Worrall,[7] a mixture of aromatic aldehyde and nitromethane in methyl alcohol solution reacts at 10–15° on addition of concd. alkali (KOH in methanol[6] or aqueous NaOH[7]) under formation of a salt of a nitroalcohol. This reaction product is converted into a nitrostyrene when its aqueous solution is added to an excess of 5 *N* HCl.

Method B.—Knoevenagel and Walter[8] found that the condensation between aromatic aldehydes and nitromethane or nitroethane proceeded at room temperature if a small amount of a primary amine was added as a catalyst. It took about one week, however, for the reaction to go to completion.

Method BH.—Hass, *et al.*,[9] shortened the reaction time to 8 hr. by boiling a mixture of aldehyde, nitroethane, amine and abs. alcohol under reflux.

Method BW.—Worrall and Cohen[10] described an even more rapid modification of the Knoevenagel procedure for the preparation of 4-dimethylamino-β-nitrostyrene and 1-(4-dimethylaminophenyl)-2-nitropropene, which required only a very short heating period.

The nature of the aldehyde to be used and whether it is to be condensed with nitromethane or nitroethane decides which of the procedures listed above is best suited in any particular case. Knoevenagel and Walter[8] reported that nitroethane did not condense with aromatic aldehydes using Method A. Furthermore, these authors observed that 4-hydroxy-3-methoxybenzaldehyde did not react with nitromethane under the conditions of Thiele's procedure, but that the expected nitrostyrene could be obtained in good yield after Method B with methylamine as catalyst. Remfry[11] found that Method A was not successful when the aldehyde had a free hydroxy group in para position. He prepared 4-hydroxy-β-nitrostyrene (IV) by saponification of 4-acetoxy-β-nitrostyrene, which was obtained by the condensation of 4-acetoxybenzaldehyde with nitromethane according to Method A. Hahn and Stiehl[12] repeated this procedure and obtained a mixture of (IV) and its acetyl derivative in a total yield of 24%.

Method BS.—Burton and Duffield[13] mentioned that the condensation of 4-hydroxybenzaldehyde with nitromethane was best effected with either methylamine or aniline as catalyst, according to unpublished data obtained in 1934 by C. W. Shoppee, but gave no details. On the basis of this suggestion, a method was worked out which produced the desired compound in 79% yield when a mixture of 4-hydroxybenzaldehyde, nitromethane and aniline was heated on the water-bath for 1 hr.

Hass, *et al.*,[9] stated that it was not possible to condense vanillin with nitroethane using Method BH. In experiments in this Laboratory, however, 1-(4-hydroxy-3-methoxyphenyl)-2-nitropropene (XL) was obtained with Method BH in a yield of 24%, while it may be prepared in 75% yield[14] under the conditions of Method B. Worrall[15] reported that attempts to condense terephthaldehyde with nitroethane failed with a primary amine as catalyst (Method B), but obtained the expected product (LIII) in "a poor yield" using triethylamine. We obtained the same product with Method BH (with *n*-butylamine) in 31% yield.

A number of arylnitroalkenes, which have not been described previously, are listed in Table I.

Antibacterial Activity.—The results of the bacteriostatic tests are summarized in Tables II and III and give the concentrations of test compounds needed to inhibit bacterial growth by 50% after an incubation time of 18 hr. Many of these data are averages of values obtained in two or three independent sets of experiments. Duplicates differed from each other by 0–5%. As a consequence of this high degree of reproducibility, one has to consider differences above 10% in the effective concentrations of two substances as significant.

All compounds tested, except one, were more effective against *Micrococcus pyrogenes* var. *aureus* (ATCC 6538), a gram-positive organism used as standard test culture in penicillin assays, than against the gram-negative *Escherichia coli* (ATCC 9637), which is used as standard in streptomycin assays. The exception referred to is 1-(2-furyl)-2-nitroethene (XXXI) which was equally effective against both organisms.

Table II shows that the addition of albumin to the culture medium reduced the effectiveness of β-nitrostyrene and its derivatives against *M. pyrogenes* only slightly, and sometimes (see, for example, XXIX) not at all. This is an important property, since loss of activity in presence of plasma proteins eliminates an antibacterial agent from consideration for the treatment of systemic infections, as was shown, for example, in an investigation of

(5a) After this report had been submitted, Dr. E. B. Hodge called our attention to the fact that U. S. Patent 2,335,384 (1943) and a paper by O. Dann and E. F. Möller, *Ber.*, **82**, 76 (1949), mention the antibacterial activity of a few nitroölefins. Furthermore, Dr. J. C. McGowan kindly sent us data on the antibacterial activity of several nitrostyrenes and related compounds taken from his Ph.D. thesis (London, 1949).

(6) J. Thiele, *ibid.*, **32**, 1293 (1899).

(7) D. E. Worrall, "Org. Syntheses," Coll. Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 413.

(8) E. Knoevenagel and L. Walter, *Ber.*, **37**, 4502 (1904).

(9) H. B. Hass, A. G. Susie and R. L. Heider, *J. Org. Chem.*, **15**, 8 (1950).

(10) D. E. Worrall and L. Cohen, THIS JOURNAL, **66**, 842 (1944).

(11) F. G. P. Remfry, *J. Chem. Soc.*, **99**, 282 (1911).

(12) G. Hahn and K. Stiehl, *Ber.*, **71**, 2154 (1938).

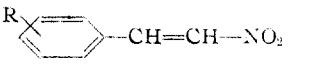
(13) H. Burton and J. A. Duffield, *J. Chem. Soc.*, 78 (1949).

(14) M. Kulka and H. Hibbert, THIS JOURNAL, **65**, 1180 (1943).

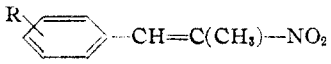
(15) D. E. Worrall, *ibid.*, **62**, 3253 (1940).

TABLE I

SUBSTITUTED PHENYLNITROALKENES



No.	R	Method	Yield, %	Recryst. solvent	M.p.,[a] °C.	Appearance	Formula	Nitrogen, % Calcd.	Nitrogen, % Found[b]
VI	2-OC_2H_5	A	26	Dil. alc.	39	Light yellow fine needles	$C_{10}H_{11}NO_3$	7.25	7.20
XV	2-OH, 5-Cl	A	12	Benzene	166–167	Deep yellow needles	$C_8H_6NO_3Cl$	7.02	6.99
XIX	2,6-Cl_2	A	74	Alcohol	66–66.5	Pale y. needles	$C_8H_5NO_2Cl_2$	6.42	6.60
XXIII	4-CH_3CONH	A	89	Alcohol	274	Bright yellow fine needles	$C_{10}H_{10}N_2O_3$	13.59	13.36
XXV	4-$(C_2H_5)_2N$	BW	70	Alcohol	98	Red prisms	$C_{12}H_{16}N_2O_2$	12.72	12.43
XXVI	4-COOH	A	97	Dil. alc.	282 dec.	Light yellow small needles	$C_9H_7NO_4$	7.25	7.25
XXVII	2-SO_3Na	A	50	Alcohol	293–294	Light yellow green plates	$C_8H_6NO_5SNa$	5.58	5.43



XXXIV	4-OH	BH[c]	29	Dil. acl.	125–125.5	Deep yellow small needles	$C_9H_9NO_3$	7.82	7.71
XXXVI	2-OC_2H_5	BH	73	Alcohol	56	Lemon y. prisms	$C_{11}H_{13}NO_3$	6.76	6.87
XXXVII	3-OCH_3	BH	54	Petrol. ether	46.5	Y. large needles	$C_{10}H_{11}NO_3$	7.25	7.12
XXXIX	2-OH, 3-OCH_3	BH	27	Dil. alc.	106–107	Lemon y. prisms	$C_{10}H_{11}NO_4$	6.70	6.60
XLI	2,3-$(OCH_3)_2$	BH	79	Dil. alc.	79–80	Light y. plates	$C_{11}H_{13}NO_4$	6.28	6.03
XLIV	2-Cl	BH	52	Dil. alc.	45	Light yellow elongated plates	$C_9H_8NO_2Cl$	7.09	6.89
XLV	2,4-Cl_2	BH	73[d]	Dil. alc.	84–85	Light yellow fine needles	$C_9H_7NO_2Cl_2$	6.04	6.14
XLVI	3,4-Cl_2	BH	52[e]	Dil. alc.	81	Light y. needles	$C_9H_7NO_2Cl_2$	6.04	5.94
XLVII	2-NO_2	BH	46[e]	Alcohol	77–78	Light y. plates	$C_9H_8N_2O_4$	13.46	13.45
XLVIII	4-NO_2	BH	55	Alcohol	114–115	Light yellow coarse needles	$C_9H_8N_2O_4$	13.46	13.38
XLIX	4-CH_3CONH	BH	30	Alcohol	132	Yellow prisms	$C_{11}H_{12}N_2O_3$	12.72	12.67
LI	4-$(C_2H_5)_2N$	BH	18	Alcohol	65	Orange platelets	$C_{13}H_{18}N_2O_2$	11.60	11.59
LII	3-CH-C(CH_3)NO_2	B[f]	67	Alcohol	108–109	Bright yellow elongated prisms	$C_{12}H_{12}N_2O_4$	11.29[g]	10.94

[a] Melting points are uncorrected. [b] Microanalysis of VI, XXIII, XXV, XXXIV–XLI, XLVII–LI by Dr. E. W. D. Huffman, Denver. Cl-substituted compounds gave low results with the micro Dumas procedure and were analyzed in this Laboratory, following a suggestion of Butler and Carter (ref. 19) by Friedrich's modification (ref. 20) of the Kjeldahl method. The same procedure was used for the analysis of compounds XXVI and XXVII. Microanalysis of LII by Dr. Carl Tiedtke New York. [c] In all preparations after Method BH *n*-butylamine was used as catalyst, except in the synthesis of XLIV where *n*-amylamine was used. [d] 24 hr. heating under reflux instead of 8 hr. [e] 12 hr. heating under reflux. [f] The preparation of this compound is described in the Experimental part. [g] Calcd.: C, 58.06; H, 4.87; Found: C, 57.76; H, 4.61.

dibromosalicil.[16] An increase in antibacterial activity on addition of albumin was observed with compounds X and XXX. Similar effects of albumin were described for a number of β-diketones.[17]

Of various substituents on the benzene ring of β-nitrostyrene, hydroxy groups in position 2 or 3 had very little effect on activity against *M. pyrogenes*, but the same group in position 4 lowered the antibacterial effectiveness, as did all other substituents in para position, with the exception of Cl (XVII, XVIII) and of a second β-nitrovinyl group (XXX). Methoxy, ethoxy or nitro groups in position 2 or 3 increased activity against *M. pyogenes*. Dichloro-β-nitrostyrenes were particularly active against the same organism, especially 2,6-dichloro-β-nitrostyrene (XIX). This compound, 1,4-bis-(β-nitrovinyl)-benzene (XXX) and 1-(2-thienyl)-2-nitroethene (XXXII) were the only 3 substances among 31 substituted β-nitrostyrenes which were more effective against *E. coli* than unsubstituted β-nitrostyrene.

Phenylnitropropene derivatives (Table III) were usually more active than the corresponding β-nitrostyrenes against *M. pyogenes* in protein-free medium, but lost a great deal of their superiority when albumin was added. Only one compound in this group—1-(3,4-dichlorophenyl)-2-nitropropene (XLVI)—was slightly more active against *E. coli* than 1-phenyl-2-nitropropene. The most active of the 55 substances tested were 1-(3,4-dichlorophenyl)-2-nitropropene (XLVI) against *M. pyogenes* in absence of albumin, 2,6-dichloro-β-nitrostyrene (XIX) against the same organism in presence of albumin and 1,4-bis-(β-nitrovinyl)-benzene (XXX) against *E. coli*. Nitromethane had no inhibitory effect on either of the two organisms in concentrations up to 112 mg./100 ml. medium. Cinnamic acid (15 mg./100 ml. medium) did not interfere with bacterial growth, indicating that the nitro group (and not only the unsaturated linkage) contributed toward antibacterial activity.

Toxicity.—The toxicity of the various arylnitroalkenes for mammals was not investigated in detail, but tests were carried out to determine the effect of

(16) O. Schales and A. M. Suthon, *Arch. Biochem.*, **11**, 397 (1946).
(17) O. Schales, *ibid.*, **34**, 56 (1951).

TABLE II

ANTIBACTERIAL ACTIVITY OF CONDENSATION PRODUCTS BETWEEN NITROMETHANE AND ALDEHYDE

No.	Compound	Concentration (mg./100 ml.) for 50% inhibition of growth *Micrococcus pyogenes* var. *aureus* 6538 Medium II	Medium II + albumin	*E. coli* 9637 Medium II
I	β-Nitrostyrene	0.57	0.69	1.02
II	2-Hydroxy-β-nitrostyrene	.58	0.72	2.12
III	3-Hydroxy-β-nitrostyrene	.58	0.59	1.37
IV	4-Hydroxy-β-nitrostyrene	1.08	1.74	1.98
V	2-Methoxy-β-nitrostyrene	0.53	0.75	5.80
VI	2-Ethoxy-β-nitrostyrene	.30	.39	>6.0[a] (no inhib.)
VII	3-Methoxy-β-nitrostyrene	.29	.45	3.14
VIII	4-Methoxy-β-nitrostyrene	.82	1.05	3.15
IX	4-Acetoxy-β-nitrostyrene	1.47	>1.8[a] (24% inhib.)	>2.4[a] (17% inhib.)
X	2-Hydroxy-3-methoxy-β-nitrostyrene	0.95	0.81	4.70
XI	4-Hydroxy-3-methoxy-β-nitrostyrene	1.43	2.00	>10.0[a] (41% inhib.)
XII	2,3-Dimethoxy-β-nitrostyrene	0.24	0.27	7.70
XIII	3,4-Dimethoxy-β-nitrostyrene	1.79	1.80	>4.0[a] (no inhib.)
XIV	3,4-Methylenedioxy-β-nitrostyrene	0.7[a]	1.1[a]	>2.0[a] (18% inhib.)
XV	5-Chloro-2-hydroxy-β-nitrostyrene	.23	0.45	1.72
XVI	2-Chloro-β-nitrostyrene	.26	.39	1.92
XVII	2,4-Dichloro-β-nitrostyrene	.12	.78	1.73
XVIII	3,4-Dichloro-β-nitrostyrene	.14	.25[a]	2.30[a]
XIX	2,6-Dichloro-β-nitrostyrene	.026	.078	0.86
XX	2-Nitro-β-nitrostyrene	.42	.46	1.02
XXI	3-Nitro-β-nitrostyrene	.47	.50	2.28
XXII	4-Nitro-β-nitrostyrene	.72	.84	1.88
XXIIII	4-Acetamido-β-nitrostyrene	>1.3[a](35% inhib.)	>1.3[a] (41% inhib.)	>1.3[a] (no inhib.)
XXIV	4-Dimethylamino-β-nitrostyrene	>1.0[a] (15% inhib.)	>1.5[a] (15% inhib.)	>1.0[a] (no inhib.)
XXV	4-Diethylamino-β-nitrostyrene	0.76	2.68	>1.0[a] (no inhib.)
XXVI	4-(β-Nitrovinyl)-benzoic acid	1.76	>3.0[a] (37% inhib.)	>3.0[a] (no inhib.)
XXVII	2-(β-Nitrovinyl)-benzenesulfonic acid (Na salt)	>20.0[a] (no inhib.)	..	>20.0[a] (no inhib.)
XXVIII	β-Nitro-α-hydrindone	1.23	5.80	6.70
XXIX	1,3-Bis-(β-nitrovinyl)-benzene	0.15	0.15	1.12
XXX	1,4-Bis-(β-nitrovinyl)-benzene	0.21	0.15	0.56
XXXI	1-(2-Furyl)-2-nitroethane	1.40	1.66	1.40
XXXII	1-(2-Thienyl)-2-nitroethene	0.71	0.97	0.89

[a] Low solubility interfered with accurate determination. Figures in parentheses indicate the degree of inhibition obtained with the concentration listed.

intravenous administration of a few selected substances on white mice. The intravenous injection of 4 compounds dissolved in propylene glycol (for technical details see[18]) and of one compound in aqueous solution, produced no harmful effects, as shown by the data given in Table IV.

Experimental

4-Hydroxy-β-nitrostyrene (IV).—A mixture of 0.1 mole of 4-hydroxybenzaldehyde, 0.1 mole of nitromethane and 1 ml. of aniline was heated on the water-bath for 1 hr. On cooling, IV appeared in form of deep yellow plates; yield 13.0 g. (79%), m.p. (from dilute alcohol) 167–168°; acetyl derivative, m.p. 161–162°. The mixed melting points with the corresponding substances prepared after Hahn and Stiehl[12] showed no depression.

2,4-Dichloro-β-nitrostyrene (XVII).—Butler and Carter[19] reported m.p. 110°. The substance obtained in this Laboratory in form of pale yellow fine needles (from alcohol) had m.p. 116–116.5°.

Anal.[20] Calcd. for $C_8H_5NO_2Cl_2$: N, 6.42. Found: N, 6.28.

3,4-Dichloro-β-nitrostyrene (XVIII).—Butler and Carter[19] reported m.p. 75°. The substance obtained in this Laboratory in the form of small yellow prisms (from alcohol) had m.p. 96°.

Anal.[20] Calcd. for $C_8H_5NO_2Cl_2$: N, 6.42. Found: N, 6.61.

4-Cyanobenzaldehyde.—Lieberman and Connor[21] mentioned in their description of the synthesis of 4-nitrobenzaldehyde from 4-nitrotoluene (with 4-nitrobenzaldiacetate as intermediate) that the same procedure, according to a private communication from L. Weisler, may be used to prepare 4-cyanobenzaldehyde. Following this suggestion, 4-cyanobenzaldiacetate was obtained in 56% yield, colorless needles (from alcohol), m.p. 109–110°.

Anal. Calcd. for $C_{12}H_{11}NO_4$: N, 6.01. Found: N, 6.04.

The diacetate was converted[21] to 4-cyanobenzaldehyde in 90% yield, colorless needles (from water), m.p. 101–102°, which in turn was hydrolyzed after Reinglass[22] in 95% yield to terephthalaldehydic acid, m.p. (in closed, CO_2 filled tube) 256°. This compound was used for the preparation of XXVI.

β-Nitro-α-hydrindone (XXVIII).—Thiele and Weitz[23] obtained this compound in attempts to prepare 1,2-bis-(β-nitrovinyl)-benzene from *o*-phthalaldehyde and nitromethane (method A) and reported m.p. 117° dec. Following this procedure, a compound resulted in 80% yield, yellow fine needles (from ligroin), m.p. 148°.

Anal. Calcd. for $C_9H_7NO_3$: C, 61.01; H, 3.98; N, 7.91. Found: C, 61.08; H, 4.11; N, 7.75.

(18) O. Schales and G. E. Mann, *Arch. Biochem.*, **18**, 217 (1948).

(19) G. E. Butler and M. E. Carter, THIS JOURNAL, **72**, 2303 (1950).

(20) E. P. Clark, "Semimicro Quantitative Organic Analysis," Academic Press, Inc., New York, N. Y., 1943, p. 41.

(21) S. V. Lieberman and R. Connor, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 441.

(22) P. Reinglass, *Ber.*, **24**, 2416 (1891).

(23) J. Thiele and E. Weitz, *Ann.*, **377**, 1 (1910).

TABLE III

ANTIBACTERIAL ACTIVITY OF CONDENSATION PRODUCTS BETWEEN NITROETHANE AND ALDEHYDES

No.	Compound	Concentration (mg./100 ml.) for 50% inhibition *M. pyogenes* var. *aureus* 6538 Medium II	Medium II + albumin	*E. coli* 9637 Medium II
XXXIII	1-Phenyl-2-nitropropene	0.087	0.51	0.95
XXXIV	1-(4-Hydroxyphenyl)-2-nitropropene	.24	0.93	1.74
XXXV	1-(2-Methoxyphenyl)-2-nitropropene	.15	1.21	8–10[a]
XXXVI	1-(2-Ethoxyphenyl)-2-nitropropene	.090	0.86	>15.0[a] (20% inhib.)
XXXVII	1-(3-Methoxyphenyl)-2-nitropropene	.065	.44	3.44
XXXVIII	1-(4-Methoxyphenyl)-2-nitropropene	.12	.55	3.67
XXXIX	1-(2-Hydroxy-3-methoxyphenyl)-2-nitropropene	.34	.99	6.05
XL	1-(4-Hydroxy-3-methoxyphenyl)-2-nitropropene	.24	1.11	6.63
XLI	1-(2,3-Dimethoxyphenyl)-2-nitropropene	.11	0.57	>10.0[a] (no inhib.)
XLII	1-(3,4-Dimethoxyphenyl)-2-nitropropene	.23	.69	>10.0[a] (no inhib.)
XLIII	1-(3,4-Methylenedioxyphenyl)-2-nitropropene	.10	.61	2.77
XLIV	1-(2-Chlorophenyl)-2-nitropropene	.043	.49	3.17
XLV	1-(2,4-Dichlorophenyl)-2-nitropropene	.010	.35	8–10[a]
XLVI	1-(3,4-Dichlorophenyl)-2-nitropropene	.007	.12	0.82
XLVII	1-(2-Nitrophenyl)-2-nitropropene	.18	.34	5.96
XLVIII	1-(4-Nitrophenyl)-2-nitropropene	.06	.23	1.34
XLIX	1-(4-Acetamidophenyl)-2-nitropropene	.29	1.02	5.54
L	1-(4-Dimethylaminophenyl)-2-nitropropene	.50	1.53	>5.0[a] (8% inhib.)
LI	1-(4-Diethylaminophenyl)-2-nitropropene	.24	1.87	2.0[a]
LII	1,3-Bis-(β-methyl-β-nitrovinyl)-benzene	.010	0.11	3.0[a]
LIII	1,4-Bis-(β-methyl-β-nitrovinyl)-benzene	.014	0.28	1.3[a]
LIV	1-(2-Furyl)-2-nitropropene	1.75	3.54	3.45
LV	1-(2-Thienyl)-2-nitropropene	0.42	1.34	1.77

[a] Low solubility interfered with accurate determination. Figures in parentheses indicate the degree of inhibition obtained with the concentration listed.

TABLE IV

LACK OF ACUTE TOXICITY OF SOME ARYLNITROALKENES

Compound	Solvent	Dose,[a] mg./kilo	Number of mice used	Number of deaths
I	Propylene glycol	80–110	15	0
XXVII	Water	190–210	18	0
XXXII	Propylene glycol	27–32	7	0
XXXVI[b]	Propylene glycol	13–15	6	0
LII	Propylene glycol	1.4–2.0	7	0

[a] The dosages used were as high as solubility of the particular compound permitted. [b] In experiments with 16 additional mice, the LD_{50} of this compound was found to be about 22 mg./kilo body weight.

1,3-Bis-(β-methyl-β-nitrovinyl)-benzene (LII).—20.1 g. of isophthalaldehyde (0.15 mole) was mixed with 27 g. of nitroethane (0.36 mole) and 1.05 g. of *n*-amylamine (0.012 mole). After short warming (water-bath at 50°) a homogeneous solution resulted which was kept at 37°. The mixture was still liquid after 7 days, but turned solid after 8 days. The reaction product was dissolved in 80 ml. of hot alcohol and yielded on cooling 25.0 g. (67%) of yellow, elongated prisms, m.p. 108–109°.

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 57.76; H, 4.61; N, 10.94.

Determination of Antibacterial Activity.—The inhibition of bacterial growth by the various test substances was determined with the aid of turbidity measurements, using a Lumetron Photoelectric Colorimeter 402-E and filter M-575. Fresh cultures, grown for 24 hr. in Medium II of Schmidt and Moyer,[24] were diluted with sterile medium (10^{-5} for *M. pyogenes* and 10^{-6} for *E. coli*) and 10-ml. portions of the diluted cultures were transferred to sterile tubes, which contained 2 ml. of water and 0.1 ml. of alcoholic[25] solution of the test substance in various concentrations. In experiments with albumin, 1 ml. of 12% beef albumin (Fraction V, Armour) in 1% NaCl solution took the place of 1 ml. of water. In each assay series there were included two control tubes, which contained the same materials as the other tubes, except that 0.1 ml. of alcohol was substituted for the alcoholic solution of the test substance.[26]

The mixtures were incubated for 18 hr. at 37° and the turbidity in each tube then was measured and expressed as percentage of full growth. This was possible with the aid of calibration curves, which relate optical density and percentage growth, and which were constructed from turbidity data of serial dilutions of the contents of the blank tubes mentioned above. The percentage growth figures were plotted against the corresponding concentrations of antibacterial agent. The resulting curve was used to find the concentration required for a 50% inhibition of growth. Examples of calibration curves and dose response curves are shown in an earlier communication.[16]

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(24) W. H. Schmidt and A. J. Moyer, *J. Bact.*, **47**, 199 (1944). This medium is clear and light in color and is, therefore, particularly suitable for photoelectric turbidity measurements.

(25) Compounds XXVI and XXVII were used in aqueous solution.

(26) This small amount of alcohol had a slight but measurable effect on bacterial growth.