3.8 (26 mg) and the disubstituted derivative (as shown by nmr) (see discussion) was obtained in hold-back volumes 8.2–12.7 (18 mg).

Acknowledgments.—The authors are indebted to Mr. A. Dornbush for the *in vitro* testing, Mr. G. Redin for the *in vivo* results, Dr. A. Tomcufcik for helpful suggestions, and Dr. R. Kelly for the radioactive labeling determinations.

Semisynthetic Penicillins. IV. Pyridylmethylpenicillins and Related Cephalosporins[1]

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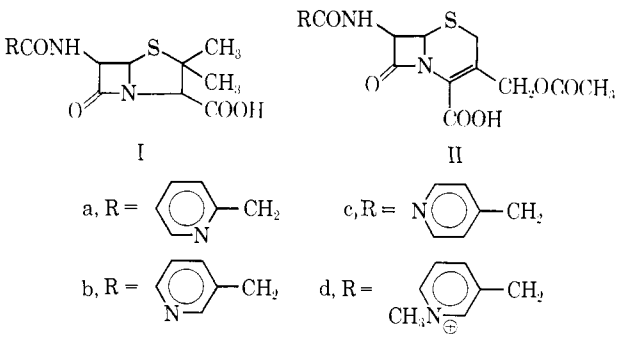
The preparation of the semisynthetic pyridylmethylpenicillins Ia–d and the related cephalosporins IIa–c is described. The *in vitro* antibacterial properties of these substances are reported. Some of them show promising broad-spectrum activity.

The work described in this paper was undertaken as part of a program designed to prepare, by chemical modification of 6-aminopenicillanic acid[2] and 7-aminocephalosporanic acid,[3] semisynthetic antibiotics with useful activity against both gram-positive and gram-negative bacteria. In the penicillin series,[4] benzylpenicillin (penicillin G) has excellent *in vitro* and *in vivo* activity against many gram-positive bacteria. However, it has only modest activity against gram-negative organisms and has rarely proved clinically useful against them. Phenoxymethylpenicillin (penicillin V) has poorer gram-negative activity than benzylpenicillin. The same is true of the sterically encumbered arylpenicillins, *e.g.*, methecillin (2,6-dimethoxyphenylpenicillin) and oxacillin (5-methyl-3-phenyl-4-isoxazolylpenicillin) which were designed to have resistance to staphylococcal penicillin lactamase. The only penicillin in clinical use with notable gram-negative activity is ampicillin, the D-α-amino derivative of benzylpenicillin. Another amino derivative of benzylpenicillin, *p*-aminobenzylpenicillin, has also been reported to have slightly enhanced *in vitro* gram-negative activity.[5] These data suggested that good gram-negative activity might be expected when the arylmethyl side chain of benzylpenicillin was retained and a basic site was incorporated into the molecule.[6] The pyridylmethylpenicillins fulfill these structural requirements. Scant data are available about structure–activity relationships in the cephalosporin series, but it has been suggested that the cephalosporin system is more amenable than the penicillin system to the development of derivatives which have gram-negative activity.[7] We therefore decided to prepare the three isomeric pyridylmethylpenicillins[8,9] and the corresponding cephalosporins.



The penicillins Ia–c and the cephalosporins IIa–c were prepared by condensing the appropriate pyridineacetic acids with 6-aminopenicillanic acid or 7-aminocephalosporanic acid. The quaternary derivative Id was obtained by treating Ib with methyl iodide. The general method of preparing semisynthetic penicillins and cephalospirins by acylation of the appropriate nucleus is well established,[10] but its application in the present case required considerable departure from the customary techniques. The pyridineacetic acids or their hydrochlorides were converted to their acid chloride hydrochlorides.[11] The use of this technique for activating the pyridineacetic acids[9] was particularly advantageous in the case of the 2 and 4 isomers since it avoided the necessity of handling these compounds in their unstable zwitterionic form.[12] The acid chlorides were coupled with 6-aminopenicillanic or 7-

(1) Part III: A. W. Chow, N. M. Hall, J. R. E. Hoover, M. M. Dolan, and R. J. Ferlauto, *J. Med. Chem.*, **9**, 551 (1966).

(2) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(3) (a) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961); (b) R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

(4) An excellent review of the more important penicillins is given by J. O. Klein and M. Finland, *New Engl. J. Med.*, **269**, 1019, 1074, 1129 (1963).

(5) A. L. Tosoni, D. G. Glass, and L. Goldsmith, *Biochem. J.*, **69**, 476 (1958).

(6) (a) A recent report by W. J. Leanza, B. G. Christensen, E. F. Rogers, and A. A. Patchett, *Nature*, **207**, 1395 (1965), that *p*-guanidobenzylpenicillin has good *in vitro* activity against the gram-negative organism *Salmonella* is in agreement with this view. (b) R. Knox, *ibid.*, **192**, 492 (1961), considers other possible determinants of gram-negative activity.

(7) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Am. Chem. Soc.*, **84**, 3401 (1962).

(8) (a) An unsuccessful attempt to prepare two of the pyridylmethylpencillins by biosynthesis has been reported by R. G. Jones, Q. F. Soper, O. H. Behrens, and J. W. Corse, *ibid.*, **70**, 2843 (1948). (b) Since the completion of this work, C. Hansch and E. W. Deutsch, *J. Med. Chem.*, **8**, 705 (1965), have suggested, on the basis of sequential analysis, that the pyridylmethylpenicillins might have good antibacterial activity.

(9) Since the completion of this work, successful use of the mixed anhydride and carbodiimide coupling procedures for the preparation of Ib and of some α-substituted derivatives of Ia and Ib has been claimed by L. C. Cheney and J. C. Godfrey, U. S. Patent 3,202,653 (1965).

(10) See, for example, J. R. E. Hoover, A. W. Chow, R. J. Stedman, N. M. Hall, H. S. Greenberg, M. M. Dolan, and R. J. Ferlauto, *J. Med. Chem.*, **7**, 245 (1964).

(11) The conditions used (PCl_5–AcCl) have been employed for converting amino acids to their acid chloride hydrochlorides. See, for example, H. Zinner and G. Brossmann, *J. Prakt. Chem.*, **5**, 91 (1957).

(12) W. von E. Doering and V. Z. Pasternak, *J. Am. Chem. Soc.*, **72**, 143 (1950).

TABLE I

ANTIBACTERIAL ACTIVITIES OF PENICILLINS AND CEPHALOSPORINS

	MIC,[a] μg/ml							
Compd[d]	*Streptococcus* C203	*S. aureus*[b]	*S. aureus*[c]	*Enterococcus*	*Proteus* sp.	*E. coli*	*K. pneumoniae*	*S. typhimurium*
Benzylpenicillin	0.013	0.049	>10	0.9	7.8	31.2	7.8	7.5
Ampicillin	0.009	0.011	>10	1.9	3.9	7.8	0.95	1.9
Ia	0.009	0.15	>10	7.8	7.8	125	7.8	15.6
Ib	0.018	0.15	>10	3.9	3.9	62.5	1.8	3.9
Ic	0.075	0.15	>10	1.9	1.9	31.2	1.9	1.9
Id	0.009	0.625	>10	3.9	31.2	31.2	15.6	15.6
7-Phenylacetamidocephalosporanic acid	0.055	0.22	1.8	31.2	15.6	31.2	15.6	31.2
Cephalothin	0.075	0.31	0.31	31.2	15.6	7.8	3.9	3.9
IIa	0.625	1.25	2.5	125	7.8	31.2	7.8	15.6
IIb	0.15	0.625	1.25	31.2	7.8	7.8	1.9	1.9
IIc	0.075	0.625	1.25	62.5	3.7	7.8	0.95	1.9

[a] Measured in broth by serial twofold dilutions. End points were determined by macroscopic readings after incubation for 18 hr at 37°. Inoculum, 10^6 organisms/ml. [b] Coagulase positive, not phage typable. [c] Finland 400, phage type 54 (penicillinase producer). [d] All compounds were tested as potassium salts, except ampicillin and Id, which were used as zwitterions.

aminocephalosporanic acid in chloroform with triethylamine as an acid acceptor.[13] The isolation of the amphoteric products was accomplished by a variety of techniques, including ion-exchange chromatography in cases where simpler approaches failed. In several instances the isolation was facilitated by destroying the residual 6-aminopenicillanic or 7-aminocephalosporanic acid by treatment with nitrous acid.[14]

The homogeneity of each of the new products was demonstrated by paper electrophoresis and the integrity of the β-lactam system was proven either by the infrared method of Hoover, *et al.*,[10] or by iodimetric titration.[15] The nmr spectra[16] of all the new compounds were consistent with the proposed structures.[17] In particular, the position of the double bond in the cephalosporins was confirmed by the presence of a two-proton signal at approximately $\delta = 3.5$ ppm, and the absence of any vinylic proton signals.[18] The three pyridylmethylpenicillins (Ia–c) were found to be remarkably stable to acid; thus at pH 2 and 25°, none of them was appreciably degraded in 2 hr.[19] This stability is presumably attributable to withdrawal of electrons from the amide oxygen atom by the electrostatic effect of the protonated pyridinium ring, which retards degradation to the penillic acid.[20,21]

The *in vitro* activities of the new penicillins and cephalosporins against a variety of gram-positive and gram-negative bacteria are reported in Table I. For comparison, the activities of benzylpenicillin, ampicillin 7-phenylacetamidocephalosporanic acid, and cephalothin (7-(2-thienylacetamido)cephalosporanic acid) are included. The activities against the gram-positive bacteria follow a more or less predictable pattern. Against *Streptococcus*, *Enterococcus*, and penicillin-susceptible *Staphylococcus* the penicillins are, generally, more active than the cephalosporins; but they are inactive against the penicillinase-producing *Staphylococcus* while the cephalosporins retain activity. None of the new derivatives is significantly more active than the respective penicillin or cephalosporin controls against gram-positive organisms, although in several cases the activities are essentially the same. However, the 3- and 4-pyridylmethyl derivatives generally satisfy the prediction of *in vitro* gram-negative activities greater than those of benzylpenicillin and 7-phenylacetamidocephalosporanic acid. While the two penicillin derivatives (Ib and Ic) have *in vitro* activities against *E. coli* equal to or slightly less than that of benzylpenicillin, both are more active against *Proteus*, *Klebsiella*, and *Salmonella typhimurium*. The 3- and 4-pyridylmethyl cephalosporin derivatives (IIb and IIc) are consistently more active than 7-phenylacetamidocephalosporanic acid against all four organisms; they are more active than cephalothin against three of the four organisms, the differences, however, being only one or two twofold dilutions. In contrast, the 2-pyridyl (Ia and IIa) and the quaternary (Id) derivatives are generally less active, having activities equal to or less than the controls.

Experimental Section

General.—Corrected capillary melting points are reported; they are not accurately reproducible because all of the compounds described melt with extensive decomposition. Qualitative and quantitative infrared spectra were obtained with a Perkin-Elmer Infracord. Electrophoreses were run on paper strips (Whatman 3MM) in a Durrum-type cell (Beckman Spinco Model R) with a potential gradient of 20 v/cm for 3 hr in a buffer consisting of 0.67% aqueous pyridine adjusted to pH 5 with acetic acid. Zones were detected by their iodine absorption,[22] and their distance of migration was measured from a glucose marker; mobilities are expressed as the ratio of the distance traveled by the compound to that traveled by penicillin G. Chloroform used in coupling experiments was freshly distilled from P_2O_5. All operations involving the penicillins and cephalosporins were performed at or below room temperature. The potassium 2-ethylhexanoate reagent was a 1.65 *N* solution of the salt in

(13) Coupling in aqueous solution gave inferior yields.

(14) See G. Cignarella, G. Pifferi, and E. Testa, *J. Org. Chem.*, **27**, 2668 (1962).

(15) "The United States Pharmacopeia," 14th Rev, Mack Publishing Co., Easton, Pa., 1950, p 429.

(16) Varian A-60 spectrometer; D_2O as solvent; Id was run as the zwitterion, all other compounds as their K salts.

(17) The protons on nitrogen, and the benzylic protons of Ia, Ic, IIa, and IIc were lost by exchange with the solvent.

(18) The ready migration of the cephalosporin double bond and the resulting changes in the nmr spectrum are discussed by J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *J. Chem. Soc., Sect. C*, 1142 (1966), and by R. R. Chauvette and E. H. Flynn, *J. Med. Chem.*, **9**, 741 (1966).

(19) These data are based on bioassays against *B. subtilis*.

(20) F. P. Doyle, J. H. C. Nayler, H. Smith, and E. R. Stove, *Nature*, **191**, 1091 (1961).

(21) The acid stability of the quaternary salt Id was not investigated, but would be expected to be equally great. The cephalosporin derivatives IIa–c should have the acid stability characteristic of the cephalosporin nucleus.

(22) R. Thomas, *Nature*, **191**, 1161 (1961).

2-propanol.[23] Air-equilibrated samples were exposed to the atmosphere (*ca.* 25° and 40% relative humidity) for several days; all other samples were dried *in vacuo* at 25° and subsequently protected from atmospheric moisture.

2-, 3-, and 4-Pyridineacetyl Chloride Hydrochlorides.—3-Pyridineacetic acid and the hydrochlorides of 2- and 4-pyridineacetic acids were obtained commercially. These materials were dried *in vacuo*, suspended in acetyl chloride (1.3 l./mole), and cooled in ice. PCl_5 (2 molar equiv) was added portionwise with stirring. The reaction mixture was stirred overnight at room temperature. It was then cooled in ice and the excess PCl_5 was destroyed by cautiously adding acetone (2 molar equiv). After a further 10 min the acid chloride hydrochlorides were collected, washed with acetyl chloride and then with ether, and dried briefly *in vacuo*. The colorless crystalline products were characterized only by their infrared spectra; bands (in Nujol mull) at 4.0 (broad), 4.8, 5.0 (NH stretching),[24] and 5.6 μ (C=O stretching). Most samples also showed a peak at 5.85 μ, attributed to carboxylic acid. The acid chlorides were used without delay.

Coupling Reactions. General Procedure.—To a suspension of 6-aminopenicillanic acid or 7-aminocephalosporanic acid in alcohol-free chloroform (5 ml/mmole) was added a measured excess of triethylamine. The solid dissolved after stirring at room temperature for *ca.* 10 min, and the resulting solution was cooled to −20° in a Dry Ice–2-propanol bath. The appropriate acid chloride hydrochloride was added portionwise, with stirring, at such a rate that the temperature was kept between −15 and −25°. After completion of the addition the reaction mixture was stirred in an ice bath for 1 hr and then evaporated under aspirator vacuum to dryness. The residue was treated as described below under the individual compounds.

2-Pyridylmethylpenicillin (Ia).—The crude product from 93 g (0.430 mole) of 6-aminopenicillanic acid, 62 g (0.323 mole) of 2-pyridineacetyl chloride hydrochloride, and 185 ml (1.32 mole) of triethylamine was dissolved in 1.5 l. of water and washed with ether. The resulting neutral solution was acidified to and maintained at pH 2 with HCl while aqueous $NaNO_2$ was added (foaming controlled by adding a few drops of ether) until a permanent positive starch–iodide test was obtained. After a further 15 min the excess nitrite was destroyed with ammonium sulfamate. The solution was brought to pH 1 with HCl and washed twice with ether. It was then brought to pH 4 with NaOH, and cooled in ice, whereupon the product crystallized as the zwitterion trihydrate (50 g, 40%), mp 91–93° dec. This material could be purified by dissolving it in water at pH 7 ($NaHCO_3$) and reprecipitating by adding HCl to pH 4. After air equilibration, it had mp 92–94° dec; infrared assay, 88%;[25] iodimetric assay, 98%.

Anal. Calcd for $C_{15}H_{17}N_3O_4S \cdot 3H_2O$: C, 46.26; H, 5.95; N, 10.79. Found: C, 46.43; H, 5.94; N, 10.93.

The unrecrystallized zwitterion trihydrate (15 g, 0.0385 mole) was dissolved in a mixture of 60 ml of potassium 2-ethylhexanoate reagent, 15 ml of 2-propanol, and 15 ml of water. The addition of a further 210 ml of 2-propanol caused the precipitation of 8.60 g (60%) of the potassium salt of the penicillin. This material could be recrystallized by dissolving it in 1:1 2-propanol–water and then adding a large volume of 2-propanol. The vacuum-dried material had mp 232–235° dec; infrared assay, 102%; electrophoretic mobility, 0.60.

Anal. Calcd for $C_{15}H_{16}KN_3O_4S$: C, 48.24; H, 4.32; N, 11.25. Found: C, 48.28; H, 4.55; N, 11.35.

3-Pyridylmethylpenicillin (Ib).—The crude product from 43.3 g (0.2 mole) of 6-aminopenicillanic acid, 29.6 g (0.154 mole) of 3-pyridineacetyl chloride hydrochloride, and 85.5 ml (0.611 mole) of triethylamine was dissolved in 500 ml of water. The neutral solution was washed with ether and treated with a solution of 36.0 g (0.10 mole) of N,N′-dibenzylethylenediammonium (DBED) acetate dissolved in 400 ml of water. The DBED salt of the product which precipitated amounted to 41.2 g (air dried). This material was dissolved in 300 ml of methanol, and 150 ml of the potassium 2-ethylhexanoate reagent was added. Addition of 650 ml of 2-propanol to this mixture caused the precipitation of 20.7 g (35% over-all) of the potassium salt of the product. This salt could be recrystallized by dissolving in 1:1 aqueous 2-propanol and adding a large volume of 2-propanol. The air-equilibrated material[26] had mp 221–223° dec; infrared assay, 96%; electrophoretic mobility, 0.46.

Anal. Calcd for $C_{15}H_{16}KN_3O_4S \cdot 0.5H_2O$: C, 47.10; H, 4.48; N, 10.99. Found: C, 46.81; H, 4.66; N, 10.84.

4-Pyridylmethylpenicillin (Ic).—The coupling reaction was carried out with 4.33 g (0.02 mole) of 6-aminopenicillanic acid, 3.84 g (0.02 mole) of 4-pyridineacetyl chloride hydrochloride, and 9.52 ml (0.068 mole) of triethylamine. The DBED salt of the product (4.05 g) was precipitated as described in the preparation of Ib. This salt was dissolved in a mixture of 12 ml of dimethylformamide (DMF) and 4 ml of the potassium 2-ethylhexanoate reagent. Addition of a further 26 ml of the potassium reagent and 300 ml of 2-propanol caused the precipitation of 2.19 g (29% over-all) of the potassium salt of the product. This material could be recrystallized by dissolving in 1:1 water–2-propanol and adding excess 2-propanol. The vacuum-dried salt had mp 221–223° dec; infrared assay, 96%; electrophoretic mobility, 0.29.

Anal. Calcd for $C_{15}H_{16}KN_3O_4S$: C, 48.24; H, 4.32; N, 11.25. Found: C, 47.91; H, 4.48; N, 11.27.

7-(2-Pyridineacetamido)cephalosporanic Acid (IIa).—The coupling reaction was carried out with 5.45 g (0.02 mole) of 7-aminocephalosporanic acid, 3.84 g (0.02 mole) of 2-pyridineacetyl chloride, and 9.52 ml (0.068 mole) of triethylamine. The crude product was dissolved in 100 ml of water, washed with ether, and the unchanged 7-aminocephalosporanic acid[27] was destroyed by nitrosation according to the procedure described in the preparation of Ia. After extraction with ether at pH 1, the aqueous solution was brought to pH 6.5 with NaOH and run through a 4.5 × 25 cm column of 100–200 mesh Bio-Rad AG 1-X8 quaternary ammonium resin in the acetate form. The column was washed successively with water, 1, 2, 5, and 10% acetic acid. Product was detected (ultraviolet) in early fractions of the 10% acetic acid eluate. These fractions were freeze dried to give 2.26 g of fluffy residue. This was dissolved in 60 ml of water, adjusted to pH 6.3 with NaOH, and mixed with a solution of 2.0 g (5.55 mmoles) of DBED acetate in 20 ml of water. The salt of the product which precipitated amounted to 1.22 g. This material was dissolved in a mixture of 6 ml of DMF and 6 ml of the potassium 2-ethylhexanoate reagent. The addition of 24 ml of 2-propanol caused the precipitation of 0.81 g (9% over-all) of the potassium salt of the product, which was recrystallized from 1:1 water–2-propanol by adding excess 2-propanol. The air-equilibrated material had mp 157–159° dec; quantitative infrared assay, 100%;[28] electrophoretic mobility, 0.50.

Anal. Calcd for $C_{17}H_{16}KN_3O_6S \cdot H_2O$: C, 45.63; H, 4.05; N, 9.39. Found: C, 45.31; H, 4.28; N, 9.53.

7-(3-Pyridineacetamido)cephalosporanic Acid (IIb).—The coupling reaction was carried out with 5.45 g (0.02 mole) of 7-aminocephalosporanic acid, 2.88 g (0.015 mole) of 3-pyridineacetyl chloride hydrochloride, and 8.40 ml (0.06 mole) of triethylamine. The DBED salt of the product (3.60 g) was precipitated as described in the preparation of Ib. This material was dissolved in a mixture of 70 ml of DMF and 25 ml of potassium 2-ethylhexanoate reagent. The addition of 170 ml of 2-propanol caused the precipitation of 1.73 g (26% over-all) of the potassium salt of the product. This material was recrystallized by dissolving in methanol, adding an equal volume of 1-propanol, and then an excess of 2-propanol. The air-equilibrated material had mp 171–173° dec; infrared assay, 98%; electrophoretic mobility, 0.44.

Anal. Calcd for $C_{17}H_{16}KN_3O_6S \cdot H_2O$: C, 45.63; H, 4.05; N, 9.39. Found: C, 45.34; H, 4.07; N, 9.48.

7-(4-Pyridineacetamido)cephalosporanic Acid (IIc).—The coupling reaction was carried out with 5.45 g (0.02 mole) of 7-aminocephalosporanic acid, 3.84 g (0.02 mole) of 4-pyridineacetyl chloride hydrochloride, and 9.52 ml (0.068 mole) of triethylamine. Isolation by nitrosation,[29] ion-exchange chromatography, and

(23) E. Jansen and H. Mückter, German Patent 965,753 (1957).

(24) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 260.

(25) In our hands, a variety of apparently pure amine salts of penicillins have given infrared assays of *ca.* 90%.

(26) Although the hemihydrate was generally obtained, some batches remained anhydrous even after air equilibration. The patent cited in ref 9 gives mp 228–230° dec for a monohydrate.

(27) Some of this material precipitated from solution at pH 2.

(28) Based on potassium 7-phenylacetamidocephalosporanate as standard. This compound gave a value of 107% when assayed against penicillin G. All of the cephalosporin derivatives described here showed an ester band at *ca.* 5.8 μ as well as the lactam band at *ca.* 5.65 μ in DMSO.

(29) In this case, the 7-aminocephalosporanic acid (2.4 g) which precipitated at pH 2 was removed before nitrosation.

precipitation of the DBED salt was carried out as in the preparation of IIa. In this case, the product was eluted from the column with 5% acetic acid and amounted to 1.25 g after freeze drying. The yield of the DBED salt was 0.93 g. This material was dissolved in a mixture of 18 ml of DMF and 6 ml of the potassium 2-ethylhexanoate reagent. The addition of 75 ml of 2-propanol caused the precipitation of 0.595 g (7% over-all) of the potassium salt of the product. This material could be recrystallized by dissolving in 1:1 water–2-propanol and adding excess of 2-propanol. The air-equilibrated material had mp 176–177° dec; infrared assay, 99%; electrophoretic mobility, 0.27.

Anal. Calcd for $C_{17}H_{18}KN_3O_6S \cdot H_2O$: C, 45.63; H, 4.05; N, 9.39. Found: C, 45.79; H, 4.10; N, 9.58.

(1-Methyl-3-pyridyl)methylpenicillin, Dipolar Ion.—To a solution of 2.5 g (6.54 mmoles) of the potassium salt of Ib in 25 ml each of methanol and water was added 5 ml of methyl iodide. After 24 hr at room temperature, the mixture was extracted several times with 1:1 ether–ethyl acetate and then with a 5% solution of dioctyl sodium sulfosuccinate[30] in ethyl acetate. The aqueous phase was stirred with a solution of 4.0 g of the sulfosuccinate in 30 ml of ethyl acetate and brought to pH 2 with HCl. The organic phase was separated and brought to neutrality (test paper) by adding triethylamine, and the yellow oil which deposited was washed (decantation) first with ethyl acetate, then with ether, and dried *in vacuo*. Trituration with DMF gave 0.70 g (30%) of the crystalline product. The material was recrystallized by dissolving in a small volume of methanol and adding several volumes of DMF and a large quantity of acetone. Vacuum-dried material[31] had mp 196–198° dec; iodimetric assay,[32] 97%; electrophoretic mobility, −0.02.

Anal. Calcd for $C_{16}H_{19}N_3O_4S \cdot 0.5H_2O$: C, 53.62; H, 5.62; N, 11.72. Found: C, 53.40; H, 5.65; N, 11.94.

Acknowledgments.—The authors wish to thank Dr. H. Winicov for some valuable suggestions which contributed to the synthetic procedures reported herein. They are indebted to Mr. J. J. Taggart for the infrared assays, to Mr. J. W. Hamill for the iodimetric assays, to Miss M. A. Carroll and her staff for the microanalytical data, and to Dr. W. E. Thompson and his staff for the nmr spectra. In the microbiological work, they had the skilled assistance of Mr. D. Ziv, Miss M. Davis, and Mr. J. Freeman.

(30) Aerosol OT. This material, which in solution constitutes a liquid cation exchanger, was used by D. A. Johnson, C. A. Panetta, and D. E. Cooper, *J. Org. Chem.*, **28**, 1927 (1963), for extraction of an amphoteric penicillin derivative.

(31) Apparently the compound scavenged sufficient water from the solvents to form a hemihydrate.

(32) The material was not sufficiently soluble in DMSO for the infrared assay. In mull it showed a strong β-lactam carbonyl stretching band at 5.69 μ.

5-Phenyl-2,4-pentadienamides as Potential Antimalarial Agents[1]

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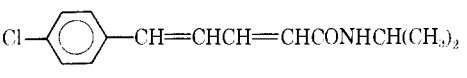
In view of the reported effectiveness of 5-(*p*-chlorophenyl)-N-isopropyl-2,4-pentadienamide against *Plasmodium gallinaceum* in the chick, various 5-phenyl-2,4-pentadienamides were prepared for evaluation against drug-resistant malarial parasites. Condensation of substituted benzaldehydes with triethyl 4-phosphonocrotonate afforded the 5-phenyl-2,4-pentadienoic acid ethyl esters. Hydrolysis with methanolic KOH gave the corresponding acids, which were converted to the acid chlorides with thionyl chloride or oxalyl chloride. Treatment of the acid chlorides with amines afforded the desired pentadienamides. None of the 5-aryl-2,4-pentadienamides was active against normal strains of *P. berghei* when administered to mice in a single subcutaneous dose of 640 mg/kg. Antimalarial studies against *P. gallinaceum* are in progress, and a satisfactory explanation is being sought for the apparent discrepancy between earlier reports and results of the current investigation.

5-(*p*-Chlorophenyl)-N-isopropyl-2,4-pentadienamide (I) is reported to be approximately four times as potent as quinine against *Plasmodium gallinaceum* in the chick and to have a therapeutic index of 12.5.[2] The structural relationships between I and chlorguanide (II) are noteworthy. The current need for an agent effective



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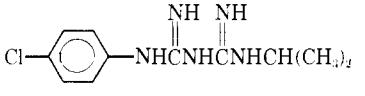

II

against drug-resistant malarial parasites[3] prompted a reinvestigation of the synthesis and biological properties of I and allied substances.[1]

The preparation of I and simple homologs thereof is not described in the literature, although there are many references to the 5-aryl-2,4-pentadienoic acid and ester precursors. The most common route to such intermediates involves the condensation of a cinnamaldehyde with malonic acid followed by decarboxylation of the intermediate product.[4] However, this process requires the preparation of a variety of substituted cinnamaldehydes, which in itself was deemed unattractive, and the over-all yields are poor.

The Reformatsky reaction has also been applied to the preparation of vinylogs of haloacetic esters. Thus *p*-chlorobenzaldehyde and ethyl γ-iodocrotonate gave 5-(*p*-chlorophenyl)-2,4-pentadienoic acid *via* the ethyl ester[5] while 3,4,5-trimethoxybenzaldehyde and methyl γ-bromocrotonate afforded the corresponding methyl ester.[6] Once again, poor yields and the known possibility of abnormal reactions on the α-carbon atom in

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(2) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents," Public Health Service Publication No. 193, Washington, D. C., 1953, pp 98, 139, 262, 276.

(3) For a recent review, see E. F. Elslager in "Annual Reports in Medicinal Chemistry, 1965," C. K. Cain, Ed., Academic Press Inc., New York, N. Y., 1966, p 136.

(4) (a) C. Liebermann, *Chem. Ber.*, **28**, 1441 (1895); (b) A. Riedel, *Ann.*, **361**, 96 (1908); (c) H. Stobbe, *Chem. Ber.*, **45**, 3396 (1912); (d) I. S. Dutt, *J. Indian Chem. Soc.*, **1**, 297 (1924–1925); (e) D. Vorlander and K. Gieseler *J. Prakt. Chem.*, [2] **121**, 247 (1929).

(5) R. C. Fuson, R. T. Arnold, and H. G. Cooke, Jr., *J. Am. Chem. Soc.*, **60**, 2272 (1938).

(6) A. S. Dreiding and R. J. Pratt, *ibid.*, **75**, 3717 (1953).