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Semisynthetic Penicillins. I. 2-Biphenylylpenicillins

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The preparation of a number of penicillins with 2-biphenylyl side chains is described. Some of the substituted 2-biphenylcarboxylic acids used as intermediates were synthesized for the first time or were prepared by new methods. These penicillins were active against penicillin G-resistant as well as penicillin G-susceptible staphylococci.

The penicillin nucleus, 6-aminopenicillanic acid, which became available in quantity as a biosynthetic product in 1959,[1] has been used as the starting material for the chemical preparation of a great number of "semisynthetic" penicillins.[2] Previously, total biosynthesis had been the only commercially feasible method for making penicillins, and, out of the limited number so obtainable, only two, penicillins G and V (benzylpenicillin and phenoxymethylpenicillin, respectively), had found wide clinical use. These two antibiotics are active against many Gram-positive organisms, including "susceptible" staphylococci, but they are not effective against the increasing number of "resistant" strains of staphylococci which are being encountered clinically. In this paper the term "susceptible" is used for those staphylococci which are sensitive to penicillins G and V, and "resistant" for those which are unaffected by high levels (*e.g.* 500–1000 γ/ml.) of these antibiotics because they have the ability to produce a lactam-opening penicillinase.[3]

The object of the work described in this and the following papers was to find a penicillin with good activity against both susceptible and resistant staphylococci as defined above. An early semisynthetic product, methicillin[4] (2,6-dimethoxyphenylpenicillin), has approximately equal activity against these two classes of organism because it is stable to staphylococcal penicillinase.[5] However, this activity is low, presumably because the same structural features confer resistance to penicillinase and also reduce the ability of the compound to interfere with bacterial metabolism. As we have previously reported,[6] 2-biphenylylpenicillin also has good stability to staphylococcal penicillinase and is considerably more active than methicillin against both susceptible and resistant staphylococci. The present paper reports an exploration of the biological effects of putting substituents on the rings of the 2-biphenylylpenicillin side chain.

The new penicillins required in this investigation were prepared by acylating 6-aminopenicillanic acid with the appropriate side chain acids. New work involved in the preparation of these acids is described in the Experimental; the footnotes to Table I cite literature references to acids which were made by known procedures. The penicillins were prepared by converting the acids to their chlorides and coupling these with 6-aminopenicillanic acid either in aqueous acetone with sodium bicarbonate as an acid acceptor[7] or, where necessary, in anhydrous chloroform in the presence of triethylamine.[4] The latter procedure was used generally with 2-biphenylcarboxylic acids bearing a substituent in the 3-position since the chlorides derived from these acids were extensively hydrolyzed in the aqueous acetone system, probably because there was less steric hindrance to the approach of a water molecule than to that of the bulky 6-aminopenicillanic acid. The mixed

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

(2) (a) Y. G. Perron, W. F. Minor, L. B. Crast, A. Gourevitch, J. Lein, and L. C. Cheney, *J. Med. Pharm. Chem.*, **5**, 1016 (1962); (b) F. P. Doyle, J. H. C. Nayler, H. R. J. Waddington, J. C. Hanson, and G. R. Thomas, *J. Chem. Soc.*, 497 (1963); (c) references to most of the relevant chemical work which has been published may be found in these two papers.

(3) Staphylococci with penicillin resistance which is not due to a lactam-opening penicillinase seem to be of less clinical importance. For a consideration of various types of penicillin resistance, see "Resistance of Bacteria to the Penicillins," A. V. S. de Reucke and M. P. Cameron, Ed., Little, Brown and Co., Boston, Mass., 1962.

(4) F. P. Doyle, K. Hardy, J. H. C. Nayler, M. J. Soulal, E. R. Stone, and H. R. J. Waddington, *J. Chem. Soc.*, 1453 (1962).

(5) G. N. Rolinson, S. Stevens, F. R. Batchelor, J. C. Wood, and E. B. Chain, *Lancet*, **2**, 564 (1960).

(6) (a) M. M. Dolan, A. Bondi, J. R. E. Hoover, R. Tumilowicz, R. C. Stewart, and R. J. Ferlauto in "Antimicrobial Agents and Chemotherapy–1961," M. Finland and G. M. Savage, Ed., American Society for Microbiology, 1962, p. 648. Subsequent papers in the same volume describe the pharmacological and clinical evaluation of this penicillin. (b) A recent publication from another laboratory [A. Gourevitch, C. T. Holdrege, G. A. Hunt, W. F. Minor, C. C. Flanigan, L. C. Cheney, and J. Lein, *Antibiot. Chemotherapy*, **12**, 318 (1962)] shows continuing interest in this compound. (c) Numerous publications have appeared comparing the biological activities of 2-biphenylylpenicillin (also referred to as "Ancillin" or SKF 12141) and other semisynthetic penicillins. See, for example, L. D. Sabath and M. Finland, *Proc. Soc. Exptl. Biol. Med.*, **111**, 547 (1962); H. Abu-Nassar, T. W. Williams, Jr., and E. M. Yow, *Am. J. Med. Sci.*, **245**, 459 (1963).

(7) Y. G. Perron, W. F. Minor, C. T. Holdrege, W. J. Gottstein, J. C. Godfrey, L. B. Crast, R. B. Babel, and L. C. Cheney, *J. Am. Chem. Soc.*, **82**, 3934 (1960).

anhydride method of coupling (using alkyl chloroformates) appeared to be inapplicable to the synthesis of penicillins in this series. Attempts to prepare 2-biphenylylpenicillin itself through the mixed anhydride of 2-biphenylcarboxylic and ethoxyformic acids, a process claimed in the patent literature,[8] yielded ethoxypenicillin. Steric factors presumably reversed the expected course of the reaction.

The new penicillins were isolated as either their potassium or their sodium salts. The former were prepared by treating the penicillin free acid in ether with potassium 2-ethylhexanoate[9] and the latter by neutralizing it in methanol with sodium methoxide. The purity of the products was estimated by measuring the intensity of the infrared absorption band at *ca.* 5.65 μ, which is characteristic of the β-lactam carbonyl group.[10] The measurement was made in dimethyl sulfoxide solution since this solvent readily dissolves the metal salts of the penicillins and does not absorb strongly in the relevant infrared region. Penicillin G was used as a standard. The assumption that the molecular extinction coefficient at this wave length is independent of the nature of the side chain was fairly well borne out by the results (Table I); most of the penicillins gave assays in the 90–105% range.

Table I reports the minimal inhibitory concentrations of the new penicillins for a susceptible and a highly resistant strain of staphylococci.[11] Although more extensive microbiological and pharmacological studies were done on many of these compounds and have been described in detail for 2-biphenylylpenicillin itself,[6] these minimal inhibitory concentrations appear to provide the most illuminating single index of their activities. The values for most of the substituted 2-biphenylylpenicillins were not very different from those for the parent penicillin (**1**), presumably because similar structural factors operate in all these compounds to protect them from attack by penicillinase without greatly impairing their antibiotic effectiveness.[12] Even compound **24**, with an additional phenyl ring in the side chain, showed little if any significant departure from the usual pattern of activities. The only readily assignable effects were seen in some of the 3-substituted derivatives. The 3-methoxy, 3-chloro, and 3-nitro compounds (**17**, **19**, **20**) had diminished activities against both types of staphylococci, probably because of excessive steric encumbrance in the neighborhood of the amide linkage and the lactam ring; the 3-methyl compound (**16**) showed this effect only marginally and the 3-fluoro compound (**18**) not at all. Rather surprisingly, the 5-nitro compound (**29**) had egregiously low activities against both organisms; the activities of the other 5-substituted derivatives (**25**–**28**) were close to the usual values. The *in vitro* susceptibilities of a number of the new penicillins to staphylococcal penicillinase were investigated. Although there was some initial degradation, the rate in all cases fell virtually to zero before much of the penicillin was destroyed; penicillin G was degraded rapidly and completely under the conditions used.[13] 2-Biphenylylpenicillin itself, although resistant to staphylococcal penicillinase, was as susceptible as penicillin G to *Bacillus cereus* penicillinase.[14] Some of the substituted derivatives were resistant to *B. cereus* penicillinase, but no systematic study was undertaken.

Although none of the derivatives described in this paper showed superiority to 2-biphenylylpenicillin itself *in vitro*, some of them may have clinical advantages which would be revealed by more intensive studies.

Experimental[15]

2′-Chloro-2-biphenylcarboxylic Acid.—A mixture of 8.01 g. (0.04 mole) of 2-chloro-2′-methylbiphenyl,[16] 7.12 g. (0.04 mole) of N-bromosuccinimide, and a pinch of benzoyl peroxide was refluxed for 2 hr. in 30 ml. of carbon tetrachloride. The reaction mixture was filtered, and the filtrate was washed with *N* sodium hydroxide and with water, dried, and evaporated to leave 2-bromomethyl-2′-chlorobiphenyl as an orange oil. This material was stirred and refluxed overnight with 14 g. of potassium permanganate in 250 ml. of water, the manganese dioxide was filtered, and the filtrate was washed with ether and acidified to give 2.6 g. (28% overall) of 2′-chloro-2-biphenylcarboxylic acid as yellow crystals, m.p. 139–141°. Recrystallization from aqueous ethanol gave colorless material, m.p. 140.5–142.5° (lit.[17] m.p. 138–139°).

Anal. Calcd. for $C_{13}H_9ClO_2$: C, 67.11; H, 3.90. Found: C, 67.24; H, 4.06.

3′-Nitro-2-biphenylcarboxylic Acid.—Crude 2-methyl-3′-nitrobiphenyl[18] (90 g.) was dissolved in 1 l. each of pyridine and water and heated on a steam bath with stirring while 400 g. of potassium permanganate was added in portions during 3 hr. Heating and stirring were continued for a further hour by which time the permanganate color had disappeared. Water (1 l.) was added, and the manganese dioxide was filtered and washed with dilute sodium carbonate. The filtrate and washings were boiled down in an open beaker to about one-third of their original volume. The volume was restored by adding water and the evaporation was repeated to complete removal of pyridine and unchanged starting material. The residue was diluted with water, washed with ether, and acidified to precipitate 85.3 g. of a light tan solid. This material was stirred at room temperature with 1.5 l. of acetonitrile and filtered.[19] The addition of 100 ml. of cyclohexylamine to the filtrate caused the rapid precipitation of 68 g. of **cyclohexylammonium 3′-nitro-2-biphenylcarboxylate** as cream colored crystals, m.p. 161–164° dec., unchanged by recrystallization from acetone.

(8) F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, U. S. Patent 2,951,839 (Sept. 6, 1960).

(9) E. Jansen and H. Mückter, German Patent 965,753 (June 19, 1957).

(10) N. H. Coy, C. W. Sabo, and B. T. Keeler, *Anal. Chem.*, **21**, 669 (1949), described a method for determining penicillin G as its procaine salt by measuring this infrared band in chloroform solution.

(11) See Table I, footnote *e*.

(12) A fuller discussion of structure-activity relationships appears in the second paper of this series [R. J. Stedman, J. R. E. Hoover, A. W. Chow, M. M. Dolan, N. M. Hall, and R. J. Ferlanto, *J. Med. Chem.*, **7**, 251 (1964)].

(13) (a) Rates were measured by the method of R. J. Henry and R. D. Housewright, *J. Biol. Chem.*, **167**, 559 (1947); see also ref. 6a and Table I, footnote *f*; (b) staphylococcal penicillinase may be inactivated by the 2-biphenylylpenicillins; *cf.* A. Gourevitch, T. A. Pursiano, and J. Lein, *Nature*, **195**, 496 (1962).

(14) J.-F. Pechère and J. Zanen, *ibid.*, **195**, 805 (1962), reported that a crude *B. cereus* preparation (as used here) contains two distinct penicillinases.

(15) Corrected capillary melting and decomposition points are reported. Infrared spectra were taken with a Perkin-Elmer Infracord and n.m.r. spectra ($CDCl_3$) with a Varian A-60 spectrometer. Only major spectral features in regions of interest are given. The pH values reported for aqueous and methanolic solutions refer to readings given by a Beckman Zeromatic pH meter with silver chloride and glass electrodes. Ethanol was S.D.A. grade 2B (anhydrous); petroleum ether was the fraction b.p. 30–60°. Dilute hydrochloric acid was used for acidification unless otherwise specified. Magnesium sulfate was used for drying organic solutions. Evaporations were carried out under aspirator vacuum.

(16) (a) M. Orchin and E. O. Woolfolk, *J. Am. Chem. Soc.*, **67**, 122 (1945); (b) attempts to oxidize this material directly to the acid with neutral permanganate or acid dichromate were unsuccessful.

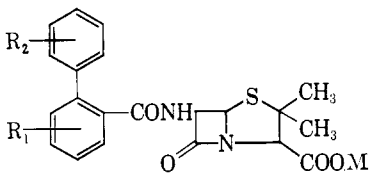
(17) H. Gilman and R. D. Gorsich, *ibid.*, **78**, 2217 (1956).

(18) D. H. Hey, A. Nechvatal, and T. S. Robinson, *J. Chem. Soc.*, 2982 (1951). The product from the Gomberg reaction of diazotized *m*-nitroaniline with toluene was distilled [b.p. 140–148° (1 mm.)], but was not further purified. It consisted of oil and crystals and was assumed to be a mixture of 2-methyl-3′-nitrobiphenyl and its isomers.

(19) The insoluble portion amounted to 26.7 g., m.p. above 250°, and was probably 3′-nitro-4-biphenylcarboxylic acid, reported m.p. 311–313° and 301° [G. W. Kenner, M. A. Murray, and C. M. B. Tylor, *Tetrahedron*, **1**, 259 (1957), and D. H. Hey and E. W. Walker, *J. Chem. Soc.*, 2213 (1948)].

TABLE I

2-BIPHENYLYLPENICILLINS



No.	R_1	R_2	Method[a]	Cryst. from[b]	Yield,[c] %	Dec. pt.,[d] °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Infrared assay, %	Minimal inhibitory concn. for staphylococci[e] (γ/ml.) Susceptible	(γ/ml.) Resistant
1[f]	H	H	A[h]	J	49	243-244	$C_{21}H_{19}N_2O_4SNa$	60.28	59.99	4.58	4.78	92	0.22	0.45
1a	H	H	...[g]	C	...	203-205	$C_{21}H_{19}N_2O_4SK$	58.04	57.85	4.41	4.65	100	0.22	0.45
2[f]	H	2'-CH_3	A[i]	E	71	...	$C_{22}H_{21}N_2O_4SK \cdot 3H_2O$	52.57	52.02	5.41	5.32	109	0.18	0.45
3[f]	H	2'-Cl	A	F	70	...	$C_{21}H_{18}ClN_2O_4SK \cdot H_2O$	51.79	51.48	4.14	4.09	103	0.09	0.45
4[f]	H	2'-NO_2	A[j]	C	54	...	$C_{21}H_{18}N_3O_6SNa \cdot 0.5H_2O$	53.39	53.66	4.05	4.29	94	3.1	0.9
5[f]	H	2'-CN	A[k]	E	75	179-180	$C_{22}H_{18}N_3O_4SK \cdot 0.5H_2O$	56.39	56.62	4.09	4.22	86	0.37	3.7
6	H	3'-CH_3	A[l]	C	22	...	$C_{22}H_{21}N_2O_4SK \cdot 1.5H_2O$	55.56	55.62	5.09	5.11	104	0.37	1.8
7[f]	H	3'-CH_3O	A	C	27	...	$C_{22}H_{21}N_2O_5SNa \cdot 0.5H_2O$	57.76	57.87	4.85	4.88	90	0.37	1.8
8[f]	H	3'-F	A	C	60	...	$C_{21}H_{18}FN_2O_4SNa \cdot H_2O$	55.50	55.70	4.44	4.57	101	0.22	0.9
9	H	3'-Cl	A	C	50	...	$C_{21}H_{18}ClN_2O_4SK \cdot 0.5H_2O$	52.77	52.44	4.01	4.03	96	0.18	0.9
10	H	3'-NO_2	A	C	52	...	$C_{21}H_{18}N_3O_6SK \cdot H_2O$	50.69	50.85	4.05	4.18	99	0.18	0.45
11	H	4'-CH_3O	A[m]	C	34	...	$C_{22}H_{21}N_2O_5SNa \cdot 1.5H_2O$	55.57	55.87	5.09	5.55	95	0.37	0.9
12	H	4'-F	A	C	50	...	$C_{21}H_{18}FN_2O_4SNa \cdot 0.5H_2O$	56.62	56.16	4.30	4.43	102	0.37	0.45
13[f]	H	4'-Cl	A[h]	D or E	45	220-222	$C_{21}H_{18}ClN_2O_4SK \cdot 1.5H_2O$	50.85	50.79	4.27	4.40	96	0.37	0.45
14[f]	H	4'-Br	A	C	59	...	$C_{21}H_{18}BrN_2O_4SK \cdot H_2O$	47.46	47.45	3.79	3.79	96	0.37	0.22
15[f]	H	4'-NO_2	A[n]	D	30	193-197	$C_{21}H_{18}N_3O_6SK \cdot H_2O$	50.69	50.26	4.05	4.32	92	0.75	0.45
16	3-CH_3	H	B	H	23	197-202	$C_{22}H_{21}N_2O_4SNa \cdot 1.5H_2O$	57.51	57.82	5.26	5.01	94	0.75	3.7
17	3-CH_3O	H	B[o]	K	25	...	$C_{22}H_{21}N_2O_5SNa \cdot H_2O$	56.64	56.44	4.97	4.90	92	3.1	7.5
18[f]	3-F	H	B	K	39	...	$C_{21}H_{18}FN_2O_4SNa \cdot H_2O$	55.50	55.53	4.44	4.50	90	0.37	0.9
19[f]	3-Cl	H	B	K	12	...	$C_{21}H_{18}ClN_2O_4SNa \cdot 1.5H_2O$	52.56	52.58	4.41	4.23	101	3.1	7.5
20	3-NO_2	H	B[p]	E	57	...	$C_{21}H_{18}N_3O_6SK \cdot 1.5H_2O$	49.79	49.73	4.18	4.48	86	6.2	1.25
21[f]	4-CH_3	H	A	I	49	...	$C_{22}H_{21}N_2O_4SK \cdot 0.5H_2O$	57.75	57.72	4.85	5.22	89	1.5	1.8
22	4-CH_3O	H	A	K	48	...	$C_{22}H_{21}N_2O_5SNa \cdot H_2O$	56.64	56.46	4.97	4.80	99	0.75	1.8
23	4-Cl	H	A[q]	C	27	...	$C_{21}H_{18}ClN_2O_4SNa \cdot H_2O$	53.56	53.59	4.28	4.37	91	0.75	1.8
24	4-C_6H_5	H	A	E	13	182-184	$C_{27}H_{23}N_2O_4SK \cdot 2.5H_2O$	58.36	58.12	5.08	4.97	99	3.1	0.9
25	5-CH_3	H	A	E	32	...	$C_{22}H_{21}N_2O_4SNa \cdot H_2O$	58.66	58.38	5.15	4.76	79	1.5	1.8
26	5-CH_3O	H	B	K	16	...	$C_{22}H_{21}N_2O_5SNa \cdot H_2O$	56.64	56.38	4.97	4.91	88	1.8	0.22
27	5-F	H	A	C	51	247-248	$C_{21}H_{18}FN_2O_4SNa$	57.79	57.80	4.16	4.27	98	0.75	3.7
28[f]	5-Cl	H	A[q]	C	29	235-239	$C_{21}H_{18}ClN_2O_4SNa$	55.69	55.64	4.01	4.30	108	0.75	1.8
29[f]	5-NO_2	H	A[r]	C	23	183-185	$C_{21}H_{18}N_3O_6SNa \cdot 2H_2O$	50.50	50.84	4.44	4.31	95	6.2	7.5
30	6-CH_3	H	A[s]	E	49	160-165	$C_{22}H_{21}N_2O_4SK \cdot 0.5H_2O$	57.75	57.72	4.85	4.94	80	0.37	3.7
31[f]	6-CH_3O	H	A[t]	C	34	...	$C_{22}H_{21}N_2O_5SK \cdot 1.5H_2O$	53.75	53.71	4.92	5.18	101	0.37	0.9
32[f]	6-Cl	H	A	C	12	...	$C_{21}H_{18}ClN_2O_4SK \cdot H_2O$	51.79	51.53	4.14	4.23	99	0.37	0.9
33	6-NO_2	H	A	C	51	...	$C_{21}H_{18}N_3O_6SK \cdot H_2O$	50.69	50.33	4.05	4.12	97	0.75	1.8
34	4-F	4'-F	A	E	65	163-165	$C_{21}H_{17}F_2N_2O_4SK \cdot 3H_2O$	48.08	48.16	4.42	4.12	108	0.37	0.45
35	4-Cl	4'-Cl	A	D	61	195-200	$C_{21}H_{17}Cl_2N_2O_4SK \cdot 1.5H_2O$	47.55	47.41	3.80	3.94	95	0.37	0.22
36	4-Br	4'-Br	A[u]	D	61	...	$C_{21}H_{17}Br_2N_2O_4SNa \cdot H_2O$	42.44	42.57	3.22	2.92	90	0.37	0.45

[a] See Experimental for description of methods A and B. [b] Recrystallizations were carried out without heating. Solvent systems: C, methanol–ether; D, methanol–isopropyl ether; E, chloroform–ether; F, acetone–ether; G, acetone–hexane; H, ethyl acetate; I, 1-butanol–isopropyl ether; J, dimethylformamide–ether; K, ethanol–ether; L, not recrystallized. [c] Yields of purified products. No attempt was made to find the maximum yield for each reaction. [d] At the decomposition point, which was very dependent on the rate of heating, the penicillin changed from a solid to a bubbling liquid; darkening and shrinking had usually occurred at a lower temperature. Where no decomposition point is reported, the same changes took place, but over a range of temperature, and neither the beginning nor the end of the change of state was well defined. [e] Measured in broth by serial twofold dilutions. Endpoints were determined by macroscopic readings after incubation for 18 hr. at 37°. Inoculum, 10^6 organisms per ml. Susceptible staphylococci: coagulase positive, not phage typable, MIC 0.05 γ/ml. of penicillin G, 1.8 γ/ml. of methicillin. Resistant staphylococci: Finland 400, phage type 54, MIC > 1000 γ/ml. of penicillin G, 3.7 γ/ml. of methicillin. [f] Susceptibility to staphylococcal penicillinase was compared to that of penicillin G manometrically (ref. 13). None of these penicillins gave more than 20% of the theoretical yield of carbon dioxide during a standard incubation period of 20 min. at 37°, and further gas evolution was negligible. Penicillin G gave an almost quantitative yield. [g] Prepared from the sodium salt. The following footnotes refer to the preparation of side chain acids: [h] See ref. 23a. [i] See ref. 15. [j] See ref. 49, but using methyl *o*-iodobenzoate and *o*-bromonitrobenzene in the Ullmann reaction. [k] 2'-Cyano-2-biphenylcarboxylic acid chloride, m.p. 77–80°, was prepared by treating diphenamic acid with thionyl chloride. H. Rapoport and A. R. Williams, *J. Am. Chem. Soc.*, **71**, 1774 (1949), give m.p. 80–81°. [l] D. H. Hey and T. M. Moynehan, *J. Chem. Soc.*, 1563 (1959). The acid was purified through its cyclohexylammonium salt, m.p. 146–149° dec. [m] See ref. 28. [n] R. L. Dannley and M. Sternfeld, *J. Am. Chem. Soc.*, **76**, 4543 (1954). An apparently simpler nitration procedure is given by D. H. Hey, J. A. Leonard, and C. W. Rees, *J. Chem. Soc.*, 4579 (1962). [o] R. Huisgen and H. Rist, *Ann. Chem.*, **594**, 137 (1955). The acid was purified through its cyclohexylammonium salt, m.p. 211–213° dec. [p] See ref. 35. [q] I. M. Heilbron, D. H. Hey, and R. Wilkinson, *J. Chem. Soc.*, 113 (1938). [r] E. K. Weisburger, *J. Am. Chem. Soc.*, **77**, 1914 (1955). [s] K. Alder, J. Haydn, K. Heimbach, and K. Neufang, *Ann. Chem.*, **856**, 110 (1954). [t] H. Richtzenhain and P. Nippus, *Chem. Ber.*, **77B**, 566 (1944). [u] C. K. Bradsher, L. E. Beavers, and N. Tokura, *J. Am. Chem. Soc.*, **78**, 3196 (1956).

Anal. Calcd. for $C_{14}H_9NO_4 \cdot C_6H_{13}N$: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.73; H, 6.65; N, 8.25.

A solution of the cyclohexylammonium salt in hot aqueous acetic acid deposited a 70% yield of 3′-nitro-2-biphenylcarboxylic acid as cream colored crystals, m.p. 154.5–156°, unchanged by recrystallization from aqueous acetic acid (lit.[20] m.p. 155–157° and 265°).

3′-Methoxy-2-biphenylcarboxylic Acid.—A solution of 90.3 g. (0.4 mole) of stannous chloride dihydrate in 450 ml. of 6 *N* hydrochloric acid was heated to 100° with stirring and 34.2 g. (0.1 mole) of cyclohexylammonium 3′-nitro-2-biphenylcarboxylate was added in portions during 30 min. The mixture was stirred for a further 30 min. at 100° until a clear solution was obtained; this was cooled, diluted with an equal volume of water, brought to pH 8 with concentrated ammonia, and filtered to remove the bulky precipitate. The filtrate was acidified to pH 3.8 to precipitate 16.5 g. of **3′-amino-2-biphenylcarboxylic acid** as a tan powder, m.p. 172–174° (Kenner, *et al.*, reported[19] m.p. 174–176°). This material was diazotized in 200 ml. of 6 *N* sulfuric acid at 0–5° with 1 equiv. of sodium nitrite, and the diazonium solution was added gradually to 2 l. of 10 *N* sulfuric acid at 100°. A brisk evolution of gas occurred. The reaction mixture was kept at 100° for 30 min. and then cooled to give a brown solid, which was dissolved in dilute sodium carbonate, warmed with charcoal, and reprecipitated with acid to yield 10.7 g. (50% over-all) of almost colorless crystals, m.p. 139–140°. Recrystallization from aqueous acetic acid gave **3′-hydroxy-2-biphenylcarboxylic acid,** m.p. 141–143°.

Anal. Calcd. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 72.76; H, 4.57.

Methylation of this material (procedure similar to that described later in the preparation of 4-methoxy-2-biphenylcarboxylic acid) gave a 66% yield of crude 3′-methoxy-2-biphenylcarboxylic acid, which was purified through its cyclohexylammonium salt, m.p. 146–148° (from acetone). The regenerated methoxy acid, colorless crystals from aqueous acetic acid, was dimorphous, m.p. 86–88° and 92.5–94° (Kenner, *et al.*, reported m.p. 88–90° for a product from methylation of noncrystalline hydroxy acid[19]).

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.89; H, 5.36.

3′-Fluoro-2-biphenylcarboxylic Acid.[21]—A solution of 5.94 g. (0.03 mole) of 1-fluorofluorenone[22] in 50 ml. of toluene was refluxed for 2 hr. over 15 g. of powdered potassium hydroxide.[23] The orange color was discharged and a light brown solid was deposited. Ice and water were added to the reaction mixture, the layers were separated, and the aqueous phase was acidified to precipitate 6.25 g. (96%) of product, m.p. 104–107°. Recrystallization from aqueous acetic acid and then from carbon tetrachloride gave colorless material, m.p. 110–112°.

Anal. Calcd. for $C_{13}H_9FO_2$: C, 72.22; H, 4.20; F, 8.79. Found: C, 71.86; H, 4.29; F, 8.70.

3′-Chloro-2-biphenylcarboxylic Acid.—3′-Amino-2-biphenylcarboxylic acid (8.52 g., 0.04 mole) was diazotized at 0–5° in 80 ml. of 6 *N* hydrochloric acid with 1 equiv. of sodium nitrite. The diazonium solution was added gradually with stirring to 12 g. of cuprous chloride dissolved in 40 ml. of 12 *N* hydrochloric acid, the temperature being kept below 10°. The mixture, which became thick with brown solid, was warmed slowly to 30–35° and kept at this temperature for 1.5 hr. while a steady evolution of gas took place. It was then boiled for a few minutes after the addition of 300 ml. of 6 *N* hydrochloric acid and cooled to give 8.17 g. (88%) of a brown powder, m.p. 121–125°. This material was dissolved in dilute sodium carbonate, warmed with charcoal, reprecipitated with acid, and recrystallized from aqueous acetic acid to give a colorless product, m.p. 124.5–126.5°.

Anal. Calcd. for $C_{13}H_9ClO_2$: C, 67.11; H, 3.90. Found: C, 66.96; H, 3.89.

4′-Fluoro-2-biphenylcarboxylic Acid.[24]—2-Fluorofluorenone (49.1 g., 0.25 mole) was treated with molten potassium hydroxide in diphenyl ether and the acidic product was separated according to a published general procedure for fluorenone cleavage.[23a] Recrystallization from aqueous acetic acid gave 12.0 g. (22%) of colorless product, dimorphous, m.p. 130–132° and 136–138°; λ_{max} for triethylamine salt as film, 11.9, 13.1, and 13.9 μ[25]; n.m.r. has a doublet centered at *ca.* 2.0 τ (J = 6 c.p.s.), both peaks showing further structure.[26]

Anal. Calcd. for $C_{13}H_9FO_2$: C, 72.22; H, 4.20. Found: C, 72.28; H, 4.14.

4′-Bromo-2-biphenylcarboxylic Acid.[24,27]—2-Bromofluorenone (25.9 g., 0.1 mole) was cleaved in the same manner as 2-fluorofluorenone. Recrystallization of the acidic product from aqueous acetic acid gave 19.4 g. (70%) of colorless material, m.p. 169–170° (lit.[28] m.p. 167–169°); λ_{max} for triethylamine salt as film, 12.0, 13.1, and 14.9 μ[25]; n.m.r. spectrum similar to that of 4′-fluoro-2-biphenylcarboxylic acid in the 2.0 τ region.[26]

Anal. Calcd. for $C_{13}H_9BrO_2$: C, 56.34; H, 3.27. Found: C, 56.29; H, 3.41.

This material was also prepared by the action of bromine on 2-biphenylcarboxylic acid.[29]

3-Methyl-2-biphenylcarboxylic Acid.—3-Amino-2-cyanotoluene[30] was acetylated by refluxing in acetic anhydride, and **3-acetamido-2-cyanotoluene,** m.p. 151–153°, was isolated by extracting the crude product in a Soxhlet thimble with petroleum ether[31] and recrystallizing the residue from 2-propanol.

Anal. Calcd. for $C_{10}H_{10}N_2O$: C, 68.95; H, 5.79; N, 16.08. Found: C, 69.05; H, 5.85; N, 16.25.

A suspension of 34.8 g. (0.02 mole) of 3-acetamido-2-cyanotoluene in 180 ml. of acetic acid and 35 ml. of acetic anhydride was treated at 0–5° with dry nitrogen trioxide to convert it to the nitroso compound, which was extracted and allowed to decompose in benzene according to a published general procedure.[32] The residue after evaporation of the benzene was distilled at 150–180° (0.2 mm.) and recrystallized from methanol to give 17.8 g. (46%) of colorless **3-methyl-2-biphenylcarbonitrile,** m.p. 73–75°.

Anal. Calcd. for $C_{14}H_{11}N$: C, 87.01; H, 5.74; N, 7.25. Found: C, 86.94; H, 5.85; N, 7.45.

This material was refluxed for 22 hr. with 12 g. of sodium hydroxide in 80 ml. of ethylene glycol and 8 ml. of water. The reaction mixture was poured into water to precipitate 19.0 g. (98%) of crude **3-methyl-2-biphenylcarboxamide,**[33] m.p. 144–

(20) (a) The method of Kenner, *et al.* (see ref. 19), gave a product identical with ours, m.p. and m.m.p. 155–157°; (b) the high melting material was reported by L. G. Makarova, M. K. Matveeva, and E. A. Gribchenko, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1452 (1958).

(21) The other possible product from cleavage of the fluorenone, 3-fluoro-2-biphenylcarboxylic acid, markedly depressed the melting point of this material.

(22) 1-Aminofluorenone was diazotized, and the diazonium hexafluorophosphate was decomposed in a procedure similar to that described in the preparation of 3-fluoro-2-biphenylcarboxylic acid. The product had m.p. 116–117°; T. L. Fletcher, M. J. Namkung, H. L. Pan, and W. H. Wetzel, *J. Org. Chem.*, **25**, 996 (1960), give m.p. 110–115°; C. G. Smith and G. O. Larson, *J. Am. Chem. Soc.*, **82**, 90 (1960), record m.p. 114–115°.

(23) (a) E. H. Huntress and M. K. Seikel, *ibid.*, **61**, 816 (1939), described a general method for the cleavage of fluorenones to 2-biphenylcarboxylic acids with molten potassium hydroxide in diphenyl ether. We used this procedure successfully on several fluorenones, but when applied to 1-fluorofluorenone it gave only a high-melting nonfluorinated product. (b) The use of solid potassium hydroxide in toluene for fluorenone cleavage was introduced by G. W. Kenner, M. J. T. Robinson, C. M. B. Tylor, and B. R. Webster, *J. Chem. Soc.*, 1756 (1962). These authors, and also E. H. Huntress and M. K. Seikel, *J. Am. Chem. Soc.*, **61**, 1966 (1939), and D. H. Hey, J. A. Leonard, and C. W. Rees, *J. Chem. Soc.*, 3125 (1963), discuss side reactions encountered during fluorenone cleavage.

(24) Formation of the 4′- rather than the 4-substituted acid was expected by analogy with the cleavage of 2-chlorofluorenone (ref. 23a) and was confirmed by the infrared and n.m.r. spectra of the product.

(25) The presence of a strong band at 13.1 μ, consistent with an *ortho*-disubstituted benzene ring, and the absence of any major band in the 14.1–14.5 μ range where a monosubstituted benzene ring should absorb confirm the 4′-halo structure. See L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 76, 77.

(26) These signals are attributed to the proton at position 3, which is coupled with those at positions 4 and 5. 2-Biphenylcarboxylic and 4′-chloro-2-biphenylcarboxylic acids give a similar pattern, while 4-chloro-2-biphenylcarboxylic acid, which lacks the proton at position 4, shows only a doublet with J = 2.5 c.p.s.

(27) C. Courtot, *Bull. Chim.* (Paris), **14**, 137 (1930), stated that a product, m.p. 165°, obtained by alkaline fusion of 2-bromofluorenone, was 4-bromo-2-biphenylcarboxylic acid. Our results cast doubt on the structure of this material.

(28) M. J. Malawski and T. Drapala, *Roczniki Chem.*, **34**, 1371 (1960).

(29) H. Winicov (private communication). The procedure was similar to that described by R. E. Buckles and N. G. Wheeler in *Org. Syn.*, **31**, 29 (1951), for the preparation of 4,4-dibromobiphenyl.

(30) J. Kenner and E. Witham, *J. Chem. Soc.*, **119**, 1452 (1921).

(31) A crystalline compound, m.p. 83–85°, λ_{max}^{Nujol} 4.48 and 5.83 μ (no NH peak), isolated from the petroleum ether extract, was probably 3-diacetylamino-2-cyanotoluene.

(32) W. E. Bachmann and R. A. Hoffman, *Org. Reactions*, **2**, 248 (1944).

(33) Only a trace of 3-methyl-2-biphenylcarboxylic acid was isolated from the reaction mixture. The amide was very resistant to both acid and alkaline hydrolysis.

148°, pure enough for conversion to the acid. Recrystallization from water gave the colorless amide, m.p. 151.5–153°.

Anal. Calcd. for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.41; H, 6.00; N, 6.76.

Nitrogen trioxide was passed for 20 min. into 10.5 g. (0.05 mole) of 3-methyl-2-biphenylcarboxamide in 75 ml. of acetic acid at 15°. The green color of the nitrogen trioxide persisted after the reaction mixture had stood overnight at room temperature. It was poured into excess 2 *N* sodium hydroxide, filtered, and acidified to precipitate 10.2 g. (97%) of 3-methyl-2-biphenylcarboxylic acid, m.p. 134–136°. Recrystallization from aqueous acetic acid and then from carbon tetrachloride gave the colorless acid, m.p. 138.5–140.5° (lit.[34] m.p. 132°).

Anal. Calcd. for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.27; H, 5.71.

3-Fluoro-2-biphenylcarboxylic Acid.—Methyl 3-nitro-2-biphenylcarboxylate[35] (25.7 g., 0.1 mole) in 250 ml. of ethyl acetate was hydrogenated at 4 kg./cm.^{2} for 1.5 hr. over 0.5 g. of 10% palladium-on-charcoal. The uptake of hydrogen was quantitative. The filtered solution was concentrated to a yellow oil, which was dissolved in 1 l. of ether, cooled in ice, and treated with hydrogen chloride gas. The **methyl 3-amino-2-biphenylcarboxylate hydrochloride** which precipitated amounted to 24.2 g. (92%), m.p. 187–190° dec. Recrystallization from ethanol–ether gave colorless material, m.p. 189–191° dec.

Anal. Calcd. for $C_{14}H_{13}NO_2 \cdot HCl$: C, 63.76; H, 5.35; N, 5.31. Found: C, 63.76; H, 5.40; N, 5.47.

This material (13.2 g., 0.05 mole) was diazotized, and the diazonium hexafluorophosphate was precipitated according to a published general procedure.[36] The hexafluorophosphate amounted to 17.2 g. (90%), m.p. 120° dec. It was added in portions to 250 ml. of boiling toluene and heating was continued for a few minutes to complete the expulsion of phosphorus pentafluoride. The cooled solution was filtered to remove some tarry material and concentrated to a dark brown oil, which was dissolved in benzene and passed through a column of activated alumina. Evaporation of the eluate gave 5.4 g. of a yellow oil. This crude methyl 3-fluoro-2-biphenylcarboxylate was hydrolyzed by refluxing with excess aqueous potassium hydroxide to give 3.2 g. (30% from methyl 3-amino-2-biphenylcarboxylate hydrochloride) of 3-fluoro-2-biphenylcarboxylic acid, m.p. 128–130°. Recrystallization from aqueous methanol gave the colorless product, m.p. 130–132°.

Anal. Calcd. for $C_{13}H_9FO_2$: C, 77.22; H, 4.20. Found: C, 72.12; H, 4.25.

3-Chloro-2-biphenylcarboxylic Acid.—A solution of 13.2 g. (0.05 mole) of methyl 3-amino-2-biphenylcarboxylate hydrochloride in 150 ml. of 0.7 *N* hydrochloric acid and 100 ml. of acetic acid was diazotized at 0–5° with 1 equiv. of sodium nitrite. The diazonium solution was run rapidly into a stirred ice-cooled solution of 12.5 g. of cuprous chloride in 125 ml. of 6 *N* hydrochloric acid, whereupon a yellow solid was deposited. The mixture was allowed to come to room temperature during 1 hr. and then heated for 5 min. on a steam bath. The solid melted, but resolidified on cooling. It was dissolved in benzene and the yellow solution was washed with dilute sodium carbonate, dried, and passed through a column of activated alumina. Evaporation of the colorless eluate gave an oil which crystallized spontaneously. Recrystallization from aqueous methanol gave 10.1 g. (82%) of **methyl 3-chloro-2-biphenylcarboxylate,** m.p. 55–57°.

Anal. Calcd. for $C_{14}H_{11}ClO_2$: C, 68.16; H, 4.49. Found: C, 68.46; H, 4.56.

3-Chloro-2-biphenylcarboxylic acid was obtained in 78% yield by hydrolyzing the ester with excess potassium hydroxide in refluxing aqueous ethanol. Recrystallization from aqueous ethanol gave the colorless acid, m.p. 184–186°.

Anal. Calcd. for $C_{13}H_9ClO_2$: C, 67.11; H, 3.90. Found: C, 66.90; H, 3.99.

4-Methyl-2-biphenylcarboxylic Acid.—1-Phenyl-1,3-pentadiene[37] (14.4 g., 0.1 mole) and 9.81 g. (0.1 mole) of ethyl propiolate were refluxed for 20 hr. in 35 ml. of toluene containing a little hydroquinone. The solvent was evaporated and the residual oil was distilled twice to give 7.7 g. of the Diels–Alder adduct, b.p. 115–125° (0.4 mm.). This material was refluxed overnight in 70 ml. of benzene with 7.96 g. (2% excess) of chloranil. The benzene solution was washed with *N* sodium hydroxide and with water, dried, and passed through an activated alumina column to remove most of the color Evaporation of the benzene and distillation of the residue gave 3.9 g. of a pale yellow oil, b.p. 126–130° (0.5 mm.). This was crystallized from petroleum ether to give 2.8 g. (12% overall) of colorless **ethyl 4-methyl-2-biphenylcarboxylate,** m.p. 71–73°.

Anal. Calcd. for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 80.30; H, 6.88.

Hydrolysis of the ester with excess refluxing aqueous sodium hydroxide gave 4-methyl-2-biphenylcarboxylic acid (quantitative yield). The acid formed colorless crystals from acetonitrile, m.p. 155–157° (lit.[34,38] m.p. 155° and 152°).

4-Methoxy-2-biphenylcarboxylic Acid.—4-Hydroxy-2-biphenylcarboxylic acid[23a] (10.7 g., 0.05 mole) was dissolved in 80 ml. of 2.5 *N* sodium hydroxide, and 50 ml. of dimethyl sulfate and 300 ml. of 2.5 *N* sodium hydroxide were added in portions, with stirring, at roughly equivalent rates during 40 min. The reaction mixture was kept at 30°. Stirring was continued at room temperature for a further 1.5 hr.; suspended solid, probably the ester of the product, was present. After the addition of 25 g. of solid sodium hydroxide, the mixture was refluxed for 1.5 hr., diluted with water, and acidified to give the crude product, which was recrystallized from aqueous acetic acid to yield 9.0 g. (79%) of material, m.p. 133–136°. Further recrystallization from the same solvent gave the colorless acid, m.p. 136–137° (lit.[39] m.p. 135°). Vacuum drying at 80° was necessary in order to obtain a sharp-melting product.

Anal. Calcd. for $C_{14}H_{12}O_3$: neut. equiv., 228.25. Found: neut. equiv., 226, 229.

2,5-Diphenylbenzoic Acid.[40]—A mixture of 20.6 g. (0.1 mole) of 1,4-diphenylbutadiene[41] and 9.8 g. (0.1 mole) of ethyl propiolate was heated at 180° for 5 hr. The cooled melt was dissolved in 150 ml. of toluene, 26.9 g. (0.11 mole) of chloranil was added, and the mixture was stirred and refluxed for 17 hr. The toluene solution was washed with 0.5 *N* sodium hydroxide and with water, dried, and evaporated. Hydrolysis of the residual ester with excess refluxing aqueous ethanolic potassium hydroxide gave 15.1 g. (55%) of the acid as colorless crystals (from benzene), m.p. 177–179° (lit.[42] m.p. 178–179°).

Anal. Calcd. for $C_{19}H_{14}O_2$: C, 83.19; H, 5.14. Found: C, 83.11; H, 4.82.

5-Methyl-2-biphenylcarboxylic Acid.—5-Methyl-2-biphenylcarbonitrile[43] (1.93 g., 0.01 mole) was refluxed for 17 hr. with 1.3 g. of sodium hydroxide in 7 ml. of ethylene glycol and 0.7 ml. of water. The mixture was poured into water, washed with ether, and brought to pH 8 to precipitate any silica which had dissolved from the reaction flask. Further acidification precipitated crude 5-methyl-2-biphenylcarboxylic acid, which was recrystallized from aqueous methanol to give 1.67 g. (79%) of product, m.p. 164–166°. A further recrystallization from carbon tetrachloride gave colorless material, m.p. 166–168° (lit.[44] m.p. 165° and 166.5–166.9°).

5-Methoxy-2-biphenylcarboxylic Acid.—5-Methoxy-2-biphenylcarbonitrile[45] (20.9 g., 0.1 mole) was refluxed for 26 hr. with 20 g. of sodium hydroxide in 100 ml. of ethylene glycol and 10 ml. of water. The mixture was poured into water, washed with ether, and brought to pH 8 to precipitate any silica which had dissolved from the flask during the hydrolysis. Further acidification caused deposition of an oil which rapidly crystallized to a brown solid. This was dissolved in 0.5 *N* sodium hydroxide, decolorized with charcoal, reprecipitated by acidification, and recrystallized from aqueous acetic acid to give 14.1 g. (62%) of colorless product, m.p. 169–173°. Further recrystallization from the same solvents gave material with m.p. 174–175.5°.[46]

(34) R. D. Haworth and P. B. Tinker, *J. Chem. Soc.*, 911 (1955).

(35) B. H. Chase and D. H. Hey, *ibid.*, 553 (1952).

(36) K. G. Rutherford, W. Redmond, and J. Rigamonti, *J. Org. Chem.*, **26**, 5149 (1961). The method used to decompose the hexafluorophosphate was a modification of these authors' general procedure.

(37) K. Alder and M. Schumacher, *Ann. Chem.*, **571**, 122 (1951).

(38) K. Alder, M. Schumacher, and O. Wolff, *ibid.*, **570**, 230 (1950).

(39) N. Chatterjee, *J. Indian Chem. Soc.*, **12**, 410 (1935).

(40) We are indebted to Dr. C. Kaiser for this preparation.

(41) R. N. McDonald and T. W. Campbell, *J. Org. Chem.*, **24**, 1969 (1959).

(42) G. R. Ames and W. Davey, *J. Chem. Soc.*, 3480 (1957).

(43) R. Ghosh, D. C. S. Pascall, and A. R. Todd, *ibid.*, 1118 (1940). The coupling of 3-acetamido-4-cyanotoluene with benzene was modified according to the general procedure of ref. 32 so that isolation of the intermediate nitroso compound was avoided. The nitrile was distilled, but no attempt was made to crystallize it.

(44) (a) K. Alder, K.-H. Decker, and R. Lienau, *Ann. Chem.*, **570**, 214 (1950); (b) N. C. Deno, *J. Am. Chem. Soc.*, **72**, 4057 (1950).

(45) (a) C. K. Bradsher and W. J. Jackson, Jr., *ibid.*, **74**, 4880 (1952); (b) the intermediate 5-nitro-2-biphenylcarbonitrile was prepared by the method of P. A. S. Smith and B. B. Brown, *ibid.*, **73**, 2438 (1951).

Anal. Calcd. for $C_{14}H_{12}O_2$: C, 73.67; H, 5.30. Found: C, 73.64; H, 5.21.

5-Fluoro-2-biphenylcarboxylic Acid.—A solution of 170 g. (0.75 mole) of stannous chloride dihydrate in 200 ml. each of 12 *N* hydrochloric acid and ethanol was heated to 50° and 44.8 g. (0.2 mole) of 5-nitro-2-biphenylcarbonitrile[55b] was added in portions, with stirring, at such a rate that the heat of reaction kept the mixture at 50–55°. This temperature was maintained for a further hour and the clear solution was then poured into 1.5 l. of ice–water to precipitate 33.3 g. (86%) of **5-amino-2-biphenylcarbonitrile,** m.p. 89–90°. Sublimation at 150° (0.3 mm.) and recrystallization from benzene–carbon tetrachloride gave colorless material with m.p. 91–92°.

Anal. Calcd. for $C_{13}H_{10}N_2$: C, 80.39; H, 5.19; N, 14.42. Found: C, 80.59; H, 4.98; N, 14.50.

A solution of 14.6 g. (0.075 mole) of 5-amino-2-biphenylcarbonitrile in 250 ml. of acetic acid was added to 5 l. of 0.6 *N* hydrochloric acid and the resulting suspension of the hydrochloride was diazotized at 3–5° with a 30% excess of sodium nitrite. To the diazonium solution was added 250 g. of sodium fluoroborate in 1.5 l. of water. The diazonium fluoroborate which rapidly precipitated amounted to 17.6 g. of cream-colored powder, m.p. 126° dec. This fluoroborate was mixed with 50 g. of sand and decomposed by intermittent heating with a flame, under aspirator vacuum, in a flask fitted with a series of Kjeldahl bulbs to trap any product entrained by the escaping gases. Gentle heating was continued for a few minutes after gas evolution had ceased. The dark residue was stirred with *N* sodium hydroxide and benzene until the tar had dissolved, and the light yellow benzene phase was washed successively with *N* sodium hydroxide, *N* hydrochloric acid (stronger acid caused separation of a black solid), and water, and concentrated to a light brown gum, which was sublimed at 130° (0.5 mm.) to give 4.9 g. of crude **5-fluoro-2-biphenylcarbonitrile** as a yellow solid, m.p. 65–75°, pure enough for hydrolysis to the acid. Recrystallization from methanol gave the colorless nitrile, m.p. 77–79°.

Anal. Calcd. for $C_{13}H_8FN$: C, 79.17; H, 4.09; N, 7.10. Found: C, 79.20; H, 4.44; N, 7.08.

The crude 5-fluoro-2-biphenylcarbonitrile (4.9 g.) was refluxed for 28 hr. with 45 ml. each of acetic acid, water, and sulfuric acid.[57] The reaction mixture was poured into water and the resulting solid was collected and stirred with 0.5 *N* sodium bicarbonate. The insoluble portion[58] was removed and the solution was acidified to give 4.45 g. (23% over-all) of 5-fluoro-2-biphenylcarboxylic acid, m.p. 106–109°. Recrystallization from aqueous acetic acid gave colorless material, m.p. 109–110° (lit.[58c] m.p. 110°).

Anal. Calcd. for $C_{13}H_9FO_2$: C, 72.22; H, 4.20. Found: C, 72.32; H, 4.45.

6-Nitro-2-biphenylcarboxylic Acid.—2-Methyl-6-nitrobiphenyl[19] (10.7 g., 0.05 mole) in 120 ml. each of pyridine and water was heated on the steam bath with good stirring, and 75 g. of potassium permanganate was added in portions during 7 hr. Filtration, evaporation, and acidification (similar procedure to that used in the preparation of 3′-nitro-2-biphenylcarboxylic acid) gave 10.4 g. (85%) of 6-nitro-2-biphenylcarboxylic acid, m.p. 187–189°. Recrystallization from acetic acid gave a cream-colored product, m.p. 189.5–191° (lit.[49] m.p. 187–188°).

6-Chloro-2-biphenylcarboxylic Acid.—6-Nitro-2-biphenylcarboxylic acid (4.86 g., 0.02 mole) in 250 ml. of ethanol was hydrogenated over 0.6 g. of 10% palladium-on-charcoal at 4 kg./cm.^{2} for 1 hr.[59] Hydrogen uptake appeared to be complete in the first few minutes. The filtered solution was evaporated to give 4.0 g. (94%) of **6-amino-2-biphenylcarboxylic acid,** m.p. 165–167°. Recrystallization from aqueous ethanol gave colorless material, m.p. 167–168°.

Anal. Calcd. for $C_{13}H_{11}NO_2$: C, 73.22; H, 5.20; N, 6.57. Found: C, 73.03; H, 5.22; N, 6.47.

Diazotization and cuprous chloride treatment of 6-amino-2-biphenylcarboxylic acid (similar procedure to that used in the preparation of 3′-chloro-2-biphenylcarboxylic acid) yielded 75% of 6-chloro-2-biphenylcarboxylic acid, m.p. 147–150°. Recrystallization from aqueous acetic acid gave a colorless product, m.p. 152–154°.

Anal. Calcd. for $C_{13}H_9ClO_2$: C, 67.11; H, 3.90. Found: C, 66.85; H, 3.73.

4,4′-Difluoro- and 4,4′-Dichloro-2-biphenylcarboxylic Acids.—The cleavage of the 2,7-dihalofluorenones (up to 0.1 mole) with fused potassium hydroxide in diphenyl ether and the isolation of the products were carried out according to the published general procedure.[23a]

4,4′-Difluoro-2-biphenylcarboxylic acid (47% yield), colorless crystals, had m.p. 124–125.5° (from aqueous ethanol).

Anal. Calcd. for $C_{13}H_8F_2O_2$: C, 66.67; H, 3.44. Found: C, 66.80; H, 3.75.

4,4′-Dichloro-2-biphenylcarboxylic acid (77% yield), colorless crystals, had m.p. 189–191° (from aqueous ethanol).

Anal. Calcd. for $C_{13}H_8Cl_2O_2$: C, 58.45; H, 3.02. Found: C, 58.55; H, 2.95.

Acid Chlorides. General Procedure.—The carboxylic acid (0.01 mole) was allowed to react with 10 ml. of thionyl chloride at room temperature. After a few hours a clear solution was usually obtained, and the excess reagent was evaporated without heating. The residue (usually a sirup) was twice dissolved in benzene and evaporated to dryness and was used without further purification. Heating or storage of 2-biphenylcarboxylic acid chlorides caused cyclization to the fluorenones.

Acylation of 6-Aminopenicillanic Acid. A.—A solution of 0.01 mole of 6-aminopenicillanic acid in 90 ml. of 3% aqueous sodium bicarbonate and 60 ml. of acetone was cooled to between −15° and −20°, stirred, and treated slowly with 0.01–0.011 mole of the appropriate acid chloride dissolved in 20 ml. of acetone. Stirring was continued for 1 hr. at −15° to −20° and for a further 3 hr. at room temperature. The reaction mixture was extracted with 300 ml. of ether in three portions, and the ether was discarded. The aqueous portion was covered with 100 ml. of fresh ether, cooled to 10°, stirred, and acidified to pH 2 with dilute hydrochloric or sulfuric acid. The layers were separated, and the aqueous portion was extracted immediately with a further 50 ml. of ether. The combined ethereal extracts were washed with 50 ml. of water in two portions and dried. The penicillin was isolated as a salt without delay.

B.—A mixture of 0.01 mole of 6-aminopenicillanic acid and 3.6 ml. (0.026 mole) of triethylamine in 25 ml. of anhydrous chloroform was cooled to 5°, stirred, and treated slowly with 0.01 mole of the appropriate acid chloride dissolved in 25 ml. of chloroform. The reaction was allowed to proceed for a further 3 hr. at room temperature. Water (50 ml.) was added, and the mixture was cooled to 10°, stirred, and acidified to pH 2 with dilute sulfuric acid. The layers were separated, and the aqueous portion was washed with 10 ml. of fresh chloroform. The combined chloroform layers were stirred at 10° with 50 ml. of water while the pH was brought to 9.0 with 5% sodium carbonate. The aqueous layer was separated, with centrifugation if necessary, and washed with 50 ml. of ether. Acidification and extraction of the penicillin into ether were then carried out as in A.

Sodium Salts of Penicillins.—The dried ethereal solution of the penicillin was evaporated without heating, and the residual sirup was dissolved in a little methanol, cooled to 5°, stirred, and titrated to pH 6.5–6.8 with methanolic sodium methoxide. The sodium salt of the penicillin was precipitated by adding several volumes of ethyl or isopropyl ether and recrystallized from a suitable solvent system (see Table I).

Potassium Salts of Penicillins.—The dried ethereal solution of the penicillin was diluted with 5 vol. of ether, cooled in ice, and treated slowly with 1 equiv. of potassium 2-ethylhexanoate, which was made up as a 30% solution in 2-propanol and diluted with several volumes of ether before use. The potassium salt of the penicillin which precipitated was recrystallized from a suitable solvent system (see Table I).

2-Biphenylylpenicillin Sodium Salt.—This compound (1)

(46) The compound described by T. Muroi, *Bull. Chem. Soc. Japan*, **34**, 178 (1961), m.p. 95–100°, was presumably impure.

(47) Hydrolysis with sodium hydroxide in refluxing ethylene glycol–water gave a product, m.p. 174–176°, λ_{max}^{Nujol} 3.08 and 4.52 μ, which was probably 5-hydroxy-2-biphenylcarbonitrile.

(48) (a) This yellow insoluble material, m.p. 128–130°, was probably 3-fluorofluorenone formed by a boron trifluoride-catalyzed cyclization during pyrolysis of the diazonium fluoroborate. It was not found in the hydrolysate of purified 5-fluoro-2-biphenylcarbonitrile. References 48b and 48c report the m.p. of 3-fluorofluorenone as 128.5–129° and 129–130°. (b) T. L. Fletcher, M. J. Namkung, W. H. Wetzel, and H. L. Pan, *J. Org. Chem.*, **25**, 1342 (1960). (c) M. J. S. Dewar and P. J. Grisdale, *ibid.*, **28**, 1759 (1963).

(49) A. M. Sadler and G. Powell, *J. Am. Chem. Soc.*, **56**, 2650 (1934). A 60% yield was obtained in the Ullmann reaction between 2-bromo-3-nitrodiphenyl and iodobenzene by using a threefold excess of the latter and carrying out the reaction in nitrobenzene as solvent. Triphenylamine was formed as a by-product.

(50) Reduction of the nitro acid with stannous chloride in hydrochloric acid gave low and variable yields of the amino compound.

had $[\alpha]^{25}D$ +252.2° (*c* 1, water); λ_{max}^{Nujol} 5.65 (lactam carbonyl), 6.0 (amide carbonyl), 13.4, and 14.2 μ (aromatic system).

2-Biphenylylpenicillin Potassium Salt.—This compound (**1a**) had $[\alpha]^{25}D$ +253.0° (*c* 1, water); λ_{max}^{Nujol} 5.65 (lactam carbonyl), 6.0 (amide carbonyl), 13.35 and 14.2 μ (aromatic system).

Ethoxypenicillin Potassium Salt.—6-Aminopenicillanic acid was acylated with ethyl chloroformate by A and the product was isolated as its potassium salt. The penicillin was obtained in 65% yield after recrystallization from dimethylformamide–ethyl acetate and had m.p. 222–223° dec.; λ_{max}^{Nujol} 5.65 (lactam carbonyl) and 5.85 μ (carbamate carbonyl); infrared assay, 103%.

Anal. Calcd. for $C_{11}H_{15}KN_2O_5S$: C, 40.48; H, 4.63; N, 8.58. Found: C, 40.40; H, 4.60; N, 8.29.

Attempted Preparation of 2-Biphenylylpenicillin by a Mixed Anhydride Reaction.—2-Biphenylcarboxylic acid (19.8 g., 0.1 mole) as its triethylamine salt in tetrahydrofuran was treated successively with 0.1 mole each of ethyl chloroformate and the triethylamine salt of 6-aminopenicillanic acid according to a published procedure.[51] Extraction of the product from acid solution into 4-methyl-2-pentanone and precipitation of its potassium salt with potassium 2-ethylhexanoate gave 25 g. of solid, m.p. 200–220° dec., showing infrared bands at 5.65 (lactam carbonyl) and 5.85 μ (carbamate carbonyl), but lacking the bands at *ca.* 13.4 and 14.2 μ attributed to the aromatic system of 2-biphenylylpenicillin. Paper chromatography[52] showed ethoxypenicillin as the major component.

Infrared Assay of Penicillins.—Measurements were made on a 0.04 *M* solution of the penicillin salt in dimethyl sulfoxide (which could contain up to 5% water) in a cell consisting of two plates of Kodak IRTRAN® AB-1 separated by a 0.1 mm. spacer. The Infracord was adjusted so that the solution showed 95% transmittance at 5.35 μ, and the peak at *ca.* 5.65 μ was traced out. The height of the peak was measured on a scale showing absorbance and compared to that given by an equimolar solution of penicillin G to find the purity of the sample; allowance was made for the hydration of the penicillins in calculating their percentage purity. Penicillin G showed a linear relation between concentration and absorbance in the 0.02 to 0.08 *M* range.

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(51) See ref. 8 for this specific reaction; ref. 7 describes the preparation of (D-α-phenoxyethyl)penicillin by a similar mixed anhydride reaction.

(52) Whatman 3MM paper, dipped in a pH 6 buffer, 0.018 *M* in citric acid and 0.064 *M* in disodium hydrogen phosphate, and dried in air; moving phase, 9 vol. of *t*-amyl alcohol and 1 vol. of 2-propanol, equilibrated with the buffer. Zones were located by spraying the paper with sodium azide and iodine, then with starch reagent. In this system, the R_f values of ethoxypenicillin and 2-biphenylylpenicillin were *ca.* 0.65 and *ca.* 0.85, respectively.

Semisynthetic Penicillins. II. Structure–Activity Studies on the 2-Biphenylyl Side Chain[1]

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A number of new penicillins were prepared in order to examine the effect of major side-chain modifications on the biological properties of 2-biphenylylpenicillin. All the new penicillins were quite active against penicillin G-susceptible staphylococci, but only those with *o*-biarylyl side chains had good activity against penicillin G-resistant staphylococci. Some of the side-chain carboxylic acids used as intermediates were synthesized for the first time or were made by new methods.

In the previous paper of this series[1] we described the preparation and *in vitro* testing of a group of substituted 2-biphenylylpenicillins. These penicillins combine high antibacterial potency with immunity to staphylococcal penicillinase; they are consequently active not only against "susceptible" staphylococci but also against the clinically important "resistant" strains. In this paper, as in the previous one, we use the term susceptible for staphylococci which are sensitive to penicillins G and V, and resistant for those which are unaffected by high levels (*e.g.*, 500–1000 γ/ml.) of these antibiotics because they produce a lactam-opening penicillinase.[2] We were interested in discovering the effect of major side-chain modifications on the biological properties of 2-biphenylylpenicillin and in elucidating the structural features responsible for its good activity against both classes of staphylococci.

The present paper reports some new semisynthetic penicillins which were prepared for this investigation. These penicillins were synthesized by condensing the appropriate side-chain carboxylic acids with 6-aminopenicillanic acid[3] using methods already described.[1] New work involved in the preparation of the side-chain acids is reported in the Experimental section; the footnotes to the tables give literature references to acids made by known procedures. Of the biological properties which were determined for the penicillins, the minimal inhibitory concentrations for two strains of staphylococci, one susceptible and the other highly resistant,[4] give the most concise indication of activity and are presented in the tables.

(1) Part I: 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).

(2) See footnote 3 of ref. 1.

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

(4) See Table I, footnote *e*. The organisms used were the same as in the previous paper (ref. 1).